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Organic Termite Repellents Tested Against *Cryptotermes Brevis* Walker

George N. Wolcott[1]

INTRODUCTION

Escape to the Tropics, to avoid the high cost of living and the complexities of modern civilization, is a mirage which becomes most apparent as lengthy residence indicates compensating disadvantages. Insidious termite attack on furniture and the interior of wooden houses in the Tropics possibly cancels out any saving from unneeded heavy clothing and other means of protection from wintry blasts and snow and ice. The West Indian dry-wood termite, *Cryptotermes brevis* Walker, is as omnipresent a symbiot of man in Puerto Rico as are cockroaches and household ants. So normal and expected is its early attack on imported furniture that many intelligent and otherwise well-informed people are convinced that it is already present in their mail-order purchases from continental sources. It is indeed true that no commercial cabinet wood of the Temperate Zone is immune from attack by dry-wood termites, but dry-wood termites do not exist where such woods are used for the construction of furniture.

Most unfortunately, the latest developments make it impossible for even local manufacturers to obtain the genuine mahogany, *Swietenia mahagoni* Jacquin, which has long been known as most resistant to infestation and thus most desirable for tropical furniture. The abundantly available Mexican or Honduran mahogany, *S. macrophylla* King, admittedly a real mahogany, is of the same genus as the endemic West Indian species, but its heartwood contains in only minute quantity the essential constituent which makes *S. mahagoni* so invaluable for permanence in the Tropics. Even more susceptible to termite attack are other woods which superficially resemble mahogany in appearance and to which this name has been applied: Philippine mahogany (so-called), *Shorea negrosensis* and other commercial species of *Shorea* and African mahogany (so-called), *Khaya ivorensis* and other commercial species of *Khaya*. Finally and most recently, Dutch mahogany, *Virola mycetis* Pulle (= *V. surinamensis* (Rol.) Warb.), locally in

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Surinam called Baboen or Baboonhoodoo, has appeared on the market in Puerto Rico. It is being extensively used by local cabinetmakers before the general public can realize the extent of the deception.

Many tropical heartwoods of the Amazon region are very resistant to termite attack, but are practically unknown and are commercially unavailable in Puerto Rico. Some native Puerto Rican woods, such as maga, sapo, palo de hierro, almendrón and guayacán, are superior to mahogany in their resistance to termite attack, but are present in such limited quantity that their extensive use in furniture construction is commercially impractical. But any wood whatsoever may be rendered absolutely, or at least superficially immune to termite attack by impregnation with suitable chemicals. To determine what these chemicals are, and which will prove to be most permanently effective in preventing attack by the West Indian dry-wood termite, *Cryptotermes brevis* Walker, is of vital economic importance to every householder in Puerto Rico. The manufacturer of furniture, both here and outside Puerto Rico, should by rights make the treatments to the termite-susceptible woods he uses before they ever reach the ultimate consumer, but the basic facts must be made available before he can take effective action. The results of extensive tests conducted by the Division of Entomology of this Station indicate which are these most effective chemicals. It is the purpose of this paper to report them.

"Graveyard tests" of stakes, using a few such chemicals for determining the effectiveness and permanence of their protection against subterranean termites, and against rot and decay, have been made elsewhere. Dry-wood termites differ so greatly in their habits and especially in their habitat, that such tests against the subterranean species are merely suggestive. Conversely, the tests of chemicals against dry-wood termites here reported must not be considered applicable to lumber exposed to the elements or buried in the soil. The dry-wood termites live under the same roof as man; they perish under exposure to heavy rain or hurricanes quite as surely as his furniture is ruined by exposure to the elements. Even if the chemicals found most permanently repellent to dry-wood termite attack are not water-soluble, their citation below is not necessarily a recommendation or even a suggestion of desirability for outdoor exposure, or underground burial.

To the sufferers from termite attack who contributed their about-to-be discarded furniture for conducting these tests, the writer is most profoundly grateful, for an abundant supply of experimental animals was quite as important as of chemicals to be tested. To those persons who contributed the often unpurchaseable experimental chemicals, he is also greatly indebted. They are: William Higburg, Reilly Tar & Chemical Corp., Indianapolis, Ind.; M. A. Warnes, E. O. Rhodes, and J. N. Roche, Koppers

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MATERIALS AND METHODS

Over 10 years have elapsed since the first tests began in Puerto Rico on the effectiveness and permanence of organic chemicals for the protection of wood against attack by the West Indian dry-wood termite, or "polilla". At that time, an abundant supply of West Indian birch lumber, or "almácigo", *Bursera* (or *Elaphrium*) *simaruba* (L.) Sarg., was available in the laboratory from another experiment, from which lathlike strips a quarter of an inch thick and an inch wide were cut, and left to air-season in the laboratory. These strips were cut into sections 1¼ inches long as they were needed for tests. These were sandpapered on all sides, and the edges and corners beveled and rounded so that any feeding of the termites would be readily apparent. "Almácigo", as compared with other woods, was subsequently assigned the low termite-resistance rating (2)[2] of 23, the lowest for any

[2] Numbers in parentheses refer to Literature Cited, p. 149.

wood being 20. Figure 1 shows how the wood blocks were tested. Thus it was considered that if any chemical could protect samples of treated "almácigo" from termite attack, it would certainly be effective when applied to any other wood with a higher termite-resistance rating.

In tests during later years, "almácigo" wood was replaced with flamboyán wood, which has a termite resistance rating of 20, the lowest of any wood readily available in Puerto Rico. Flamboyán, or "Royal Poinciana", *Delonix* (or *Poinciana*) *regia* (Bojer) Raf., has been extensively planted along the roads of Puerto Rico, as well as just inside the boundary wall of

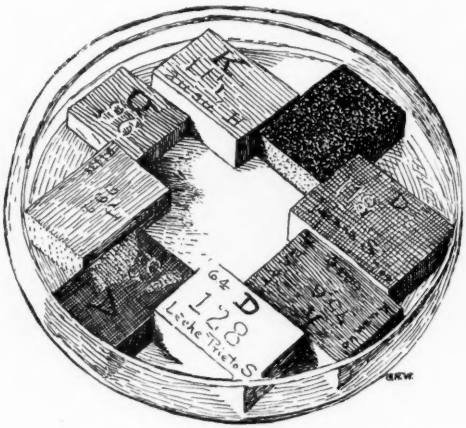

FIG. 1.—Testing wood samples for termite resistance. Drawn by G. N. Wolcott. (Original.)

the house of the writer, because of the magnificent display of its flowers during the summer. Its wood had no commercial value, and if it were not possible to induce a "caminero" (highwayman or road-tender) to contribute a section of the trunk of a flamboyán tree which he had cut down for firewood for heating a drum of tar to be used in road repairing, one could always sacrifice a tree out of one's own yard. Thus an extensive and easily renewed supply of wood blanks of a most termite-susceptible wood was assured for the entire further course of the tests.

The first important chemical tested was pentachlorphenol. Because it was first, tests in subsequent years with other effective chemicals can never catch up with it in proof of its superior permanence. The large sample of the chemical, which rather unpromisingly looked like poorly ginned cotton seed, contributed by the Monsanto Chemical Co. of St. Louis, Mo., some

years previously, was opened on January 18, 1944, and 1 gm. was weighed out to make a 2-percent solution in 50 cc. of benzol.

Three air-seasoned blanks of almácigo were given a final sandpapering before the one marked in India ink "2-percent pentachlorphenol" was submerged in the liquid. To ensure complete submergence the container for the liquid was only slightly more than an inch in diameter, and the almácigo blank was tied to the end of a glass rod with a cotton string so that its lower end rested on the bottom of the container, and its top end was half an inch or more below the top level of the liquid. The glass rod could be twirled between the fingers to dislodge any air bubbles from the submerged wooden block both at the beginning and at the end of the 10-minute period of complete submergence. After the first block had been impregnated with pentachlorphenol at 2 percent for 10 minutes and removed from the solution, this was diluted to 1 percent, the sample marked "1-percent pentachlorphenol" was placed in the liquid, and subsequently a block marked "0.5-percent pentachlorphenol" was immersed in a further dilution. The treated blocks of wood removed from the liquid after 10 minutes submersion were placed on the sill of an open window to dry in the sunshine, the concavity of the painted metallic sill accidentally being so formed that only two corners and an edge of the block were required for contact to hold it firmly in place for the free circulation of the air around it while the solvent evaporated. An hour or more after being thus exposed in the sunshine the treated blocks were placed on end in a petri dish and put on a top shelf until the following day (fig. 2).

The size of the blocks of wood had been carefully planned so that nine of them would just fit when arranged flat in a 145 x 15 mm. petri dish, using eight treated blocks, and if desired, in the center, one of West Indian mahogany (*Swietenia mahagoni* Jacquin), which has a termite-resistance rating of 80, for check. (See fig. 1.)

On January 20, 1944, the 1-percent-pentachlorphenol block was placed with other treated samples and mahogany in such a petri dish, together with numerous fresh termites removed from an infested desk. The petri dish was wrapped in a towel and placed on a shelf in a windowless constant-temperature oven set at 90°F. By the following day, most of the termites were on the mahogany, getting as far away as possible from the 1-percent pentachlorphenol-treated sample. On January 22, 22 termites were dead, their dead bodies being mostly on or around the pentachlorphenol block, which very obviously was toxic to them at that time and concentration of the chemical.

Then a fresh solution was prepared and almácigo blocks were impregnated at 0.2 percent and 0.1 percent. By January 29, the 0.1-percent-treated sample had been attacked by termites, and by January 31, so had the one

treated with the 0.2 percent of pentachlorphenol. But, although repeatedly tested, it was not until the following October that the 0.5-percent pentachlorphenol-treated sample was attacked. On December 21, 1944, the sample treated with 1 percent of pentachlorphenol showed signs of being eaten, but was left in place in the test so that the gouge might become unmistakable. On January 6, 1945, the sample was removed from the test, although the gouge was not much deeper. It has been repeatedly tested since, but although termites may rest on it in numbers when no other sample is more attractive, none has eaten it in the succeeding 9½ years. Long ago its entire back side has been filled with the penciled dates on

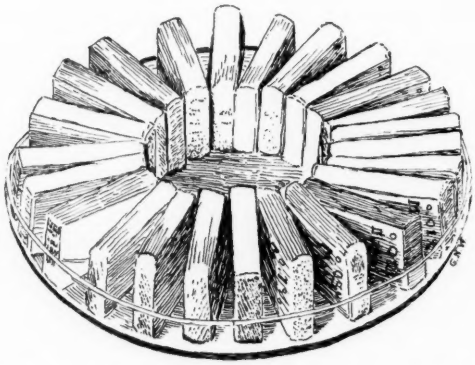

FIG. 2.—Method of stacking blocks of wood after impregnation to ensure maximum exposure for volatilization of chemicals. Drawn by G. N. Wolcott. (Original.)

which it was removed from tests during which it had not been eaten. Thus, so far as the West Indian dry-wood termite is concerned, it may be considered that submersion in a 1-percent solution of pentachlorphenol should give almost perfect protection against attack to any kind of wood, and that the 5-percent solution commercially used has a very ample margin of error for the application of this chemical.

The steps taken in testing pentachlorphenol have been related in detail not only because of the intrinsic importance of this chemical, but because a somewhat similar perfected procedure was adopted as routine in the testing of other chemicals, with possible minor improvements in technique as the investigation proceeded. Thus, initial impregnations at 2, 1, 0.5, 0.2, 0.1, and 0.05 percents were standard for the great bulk of the chemicals from which deviation was made only when the very small sample of chemical available for testing made necessary the use of an initial solution at a

smaller concentration, and greater dilutions to 0.02, 0.01, 0.005, and in some cases, even 0.002 percent.

The smoothest and most polished side of the wooden block was used for labeling. The label was written in India ink just before submergence in the solution of the chemical. The termites ate or disregarded the label in India ink as though it were not there, thus its presence in no way affected the results of the tests. The label as written at the top of the front face of the block showed the concentration of the chemical. Below that came the name of the chemical, and finally towards the bottom, the date of treatment, the back side of the block being reserved for later pencil notations of the date on which the sample was attacked, or the dates when it was temporarily removed from the tests uneaten. It was found desirable to have the writing of the label cover as much of the face of the block as possible, for in this way even the smallest disturbance of the letters or figures at once became apparent. Such very slight attacks were ordinarily not considered final or conclusive. To make sure of a major gouge or gouges, or tunnel or incipient tunnel, being eaten by the termites, the sample was continued under test until there could be no doubt that it had been attacked. That all the termites were noted resting on a sample was by no means convincing evidence that they were eating it: The block must show a definite gouge or tunnel, preferably through some letters or figures of the label.

The same glassware, the same glass rods, the same kind and size of wood blocks for impregnation, the same time and procedure for impregnation and subsequent exposure of samples for volatilization of the solvent, and subsequently of the much slower volatilization of the chemical itself when exposed in the air, were used for all chemicals that the results might be entirely comparable. Possibly a longer time of exposure, and especially pressure impregnation, would show a longer period of resistance to termite attack, and perhaps more closely approximate commercial procedure. But from the standpoint of prompt elimination of valueless chemicals, the minimum impregnation shortened the total time necessary for completing the investigation, and gave a fair, and it is hoped an impartial, standard of comparison for all the chemicals tested.

The problem of keeping so many small wood samples amply exposed to the air, so that volatilization of the chemical itself from the sample might be comparable to that which might be anticipated from furniture or interior trim, proved to be more difficult. For several weeks after the initial impregnation of a series of wood samples with a chemical, all uneaten samples (except those being tested) were left standing on edge in circles buttressed against the side of the petri dish and looking like a miniature Stonehenge (see fig. 2). But they could not be kept thus indefinitely.

The too zealous activities of the laboratory janitor made such exposure

impossible. The samples were too easily toppled over like rows of dominoes, and the petri dishes and shelf space were needed for the fresh series of samples treated with other chemicals. Eventually, those uneaten were laid away in termite-infested insect-display cabinets, the most heavily impregnated by each chemical on the bottom of the pile, and the least impregnated on top. The glass-topped display cabinets had been so largely destroyed by insect attack as to be entirely gone on one side; thus the free circulation of outside air around the sample was not impeded, even if it were not exactly stimulated.

As attacked samples accumulated, the initial small insect-display cabinet, which had been ample to contain them at first, was replaced with one very much larger, and as this became termite-infested, its contents furnished a supply of live termites for experimentation when all other sources, such as discarded furniture of friends and family, failed. The eaten blocks of wood, once the very thin, outer layer of chemical impregnation had been broken and passed, were, of course, as desirable to the termites, and as susceptible to attack, as untreated almácigo or flamboyán lumber. Moreover, one can shake the termites from them into an open petri dish without mechanical injury quite as easily as from infested plywood sheets of infested radio cabinets, and much more easily than from the wooden members of furniture that has to be chopped apart with a machete. The hollowed-out wood samples when removed from the large insect-display cabinet formed readily portable examples of termite injury that could be presented to interested visitors, or used for sending insect specimens or individual glass vials of insects as airmail enclosures.

The selection of additional chemicals for test was a matter of development as experience or accident over the years seemed to indicate profitable leads, and from the very nature of the investigation could not have been planned in advance. We commenced by testing the pure constituents of coal-tar creosote, contributed by Reilly and Koppers, none of which proved to be as effective as the crude creosote itself. Numerous chlorinated and other phenols were tested for comparison with pentachlorphenol, some purchased from Eastman, but others contributed by Monsanto and Dow. The experimental laboratories of other manufacturers, such as Laurel Hill of General Chemicals, and Whitemarsh of Penn Salt, hopefully submitted numerous samples of experimental chemicals. Because of their resistance to the attack of marine borers, many naphthenates and other compounds of naphtha were tested, mostly submitted by Nuodex Products of Elizabeth, N. J. When weed-killers were first being tested in Puerto Rico, A. S. Crafts of the Davis, Calif. Agricultural Experiment Substation, suggested trying some of the dinitro compounds that he had found most toxic to plants. All the new insecticides were tested before they had been named and while

they were still represented merely by manufacturers' code numbers, despite full recognition of the fact that the qualifications for effectiveness and permanence in impregnating woods to repel the attack of termites are quite different from those for insecticides.

As tests for many years previously had been and were continually being conducted on the natural resistance of woods to termite attack, it appeared logical that an important source of termite-repellent chemicals should be one or more extractives of the woods known to be termite resistant, or similar compounds. This presumption, based on the proved value of β-methylanthraquinone, or "tectoquinone", an extractive of East Indian teak, *Tectona grandis* L., turned out in this case to be a blind alley, for not one of the commercially available quinones tested, and only a few of their metallic lakes prepared by Yolanda Pirazzi of the laboratory of the Department of Chemistry, proved to be as termite-resistant as tectoquinone itself. All of the other extractives of wood which could be obtained from commercial sources, proved to be valueless.

At this juncture, the whole-hearted cooperation of Holger Erdtman of the Royal Institute of Technology, Stockholm, Sweden, made successful progress possible. Other European researches had demonstrated the toxicity to wood-decaying fungi of the extractives he was obtaining from coniferous woods, and he generously contributed ample samples of these experimental chemicals for tests with termites. Furthermore, being familiar with what other widely separated chemists were accomplishing in the extraction and identification of wood constituents of deciduous trees, he sent a sample of chlorophorin prepared by F. E. King of Nottingham University, England, from a species of *Chlorophora* similar to the dye-wood "mora" of Puerto Rico. He suggested to M. L. Wolfrom of Ohio State University that the extractives he was obtaining from Osage orange, *Maclura pomifera* (Raf.) Schneider, should be tested for termite attack, as our tests with the wood itself had shown it to be the only termite-resistant wood of all Temperate Zone North America, excepting only very gummy southern cypress.

RESULTS

BASIS OF COMPARISON

After having considered several possible arrangements for presentation of results, and having discarded tables, the alphabetical method, or grouping by chemical affinity, as used in a previous publication (3), it seemed desirable to be guided mainly by the permanent effectiveness of the chemical in protecting wood from termite attack. Those chemicals least valuable for the purpose are listed first, and gradually as the end of this section is approached, those which seem most desirable are discussed in greater detail.

This is simple enough at first, but subsequently becomes more compli-

cated. For instance, many chemicals were unavailable in quantities that could be used for impregnation at concentrations of 0.5, 1, or 2 percent, and it is impossible to extrapolate with any great confidence what unexpected qualities they might have displayed at greater concentrations. Yet, because any chemical that continues to render the block continually resistant at a 0.5-percent dilution is by that much superior to pentachlorphenol, that has been made the point of division. Blocks impregnated with pentachlorphenol were eaten at 0.5 percent, and after an initial gouge was made in the sample impregnated with 1-percent pentachlorphenol, they later proved continuously resistant. From the standpoint of termite resistance all chemicals are judged as "not so good as pentachlorphenol", or the very special few "apparently better than pentachlorphenol".

THE LESS EFFECTIVE CHEMICALS

As was indicated by the reactions of the termites attacking wood impregnated with some of the least resistant chemicals, such common solvents as petroleum ether, benzol, alcohol, acetone, and water volatilized so completely within 24 hours after submersion that all traces of them completely disappeared. Pyridine, a coal-tar product supplied by Reilly, sometimes used as solvent, did not, however, volatilize so rapidly, and 3 days elapsed before the termites started eating the sample of almácigo wood submerged in 100-percent pyridine. Its characteristic odor, so offensive to us, may not be apparent at all, or in the same way, to the termites. Indeed, one should beware of judging anthropomorphically the value of any suggested termite-repellent on the basis of how horrible its odor may seem to man's senses of taste or smell. Of other liquid coal-tar constituents contributed by Reilly, 2,6-lutidine protected wood 4 days, and pure α-picoline only 5 days. Commercial phenol, or carbolic acid, protected the sample in which it was submerged for 32 days, but that dipped in 2-percent phenol was eaten the following day. A sample impregnated with 2 percent of 2,6-bromophenol was eaten in 5 days, and with *p*-bromophenol in 7, but the 2,4-bromophenol protected it 16 days. Submersion in a sample of undiluted liquid orthochlorphenol, submitted by Monsanto for comparison with their pentachlorphenol, protected a sample against termite attack for 13 days.

Plastic cellulose acetate proved to be more attractive to the termites than the wood on which its film was deposited, the 2-percent film being eaten off in 2 days, with the wood underneath untouched so long as there was any more plastic to eat. Both styrene dibromide and polyvinyl acetate (duPont) were also more attractive than almácigo wood, and the plastic polystyrene, contributed by Dow, at 2 percent resisted attack only 12 days. Dow's Saran, a polymerized vinylidene chloride, produced a much more termite-

resistant film, which applied undiluted at 20 percent, was not broken through by the termites for 1 year and 10 months.

Pine Oil No. 91 of the American Turpentine and Tar Co., New Orleans, La., gave no protection against termite attack, the samples with a 2-percent impregnation being eaten in 2 days. A sample impregnated with cedar oil (Central Scientific) at 2 percent was eaten in 3 days. Yarmor No. 302 pine oil of Hercules Powder Co., and their α-terpineol, at 2 percent protected samples for 6 days, but submerging in the latter undiluted protected for 92 days.

Of the solid coal-tar constituents contributed by Reilly, the 2-percent impregnations of quinoline, chrysene, and *m*,*p*-cresol were attacked in 7 days. Acenaphthene, fluorene, and carbazol, contributed by Koppers, and 1,3,5-xylenol of Reilly at 0.5 percent protected the blocks for only 3 to 5 days; at 2 percent, the carbazole-impregnated block was eaten in 14 days, the one impregnated with 1,3,5-xylol in 54 days, with acenaphthene in 62 days, while the fluorene repelled attack for 73 days. The following chemicals at a 0.5-percent impregnation were effective only for 5 days: 1,2-naphthoquinone, indigotin, 4,4′-dihydroxy-diphenyl, Reilly's Transote, sucrose octoacetate—a very bitter sugar from Niacet Chemical Corp. of Niagara Falls, N. Y., copaivic acid, diphenylene oxide (Reilly), diphenyl, styrene or vinyl benzene, and thiophenyl; and for 7 days: 2,6-dibromo-4-aminophenol, catechol, *p*-chloracetophenone, 9,10-dichloranthracene, the wood-constituent gambier contributed by J. S. Young & Co., Baltimore, Md., naphthalene tetrachloride, Koppers' phenanthrene, and hexachlorphenol.

Hexachlorphenol, which at 1 percent protected wood for 133 days, was effective at 2 percent for 10 years after impregnation. Thus, the additional chlorination, instead of making it more termite-repellent than pentachlorphenol, merely had the effect of requiring a greater concentration to be equally repellent. For comparison, blocks impregnated with pentabromophenol at 0.5 percent were eaten in 14 days, but the wooden block impregnated with 1 percent is still uneaten 10 years later.

Three experimental chemicals submitted by the Laurel Hill Laboratory of General Chemical Co.: Hexachlordiphenyl trisulfide, phenol monochlor potassium acetaldehyde, and dichloro-dihydro-anthracene, with which wood samples were impregnated at 0.5 percent afforded protection for only 8 or 9 days; the samples showed little greater resistance to termite attack at greater concentrations.

The following constituents of woods imparted so little resistance to termite attack that almácigo or flamboyán wood samples impregnated with them at a concentration of 0.5 percent were eaten in 8 to 10 days: Morin; pyrogallol; cashew nut-shell oil or Cardol, presented by Louis

Dejoie of Haiti; copper anacardate, prepared by the laboratories of General Foods; Terposol No. 8 and Hercolyn, a hydrogenated methyl ester of abietic acid submitted by Hercules Powder Co.; fustic, contributed by J. S. Young & Co. of Baltimore, Md.; resorcinol, and vanillin. Not one imparted much greater resistance at 1 or 2 percent, and all may be considered valueless in repelling termites. Indeed, Bengt Leopold, working in Erdtman's Organic Chemistry Laboratory of the Royal Institute of Technology at Stockholm, Sweden, found that the dark, amber-colored excrement of drywood termites which had been eating Douglas fir contained 13 percent of vanillin, and estimated that if such a vanillin-rich excrement were available in commercial quantities it would be worth $50 a ton as a source of vanillin!

When the gamma isomer of benzene hexachloride or hexachlorocyclohexane was not commercially available, a mixture of the alpha and beta isomers could be obtained from Eastman. This mixture of the isomers at 0.5 percent prevented termite attack for only 10 days, at 1 percent for 15, and at 2 percent for 44. The gamma isomer, supplied by duPont when it was still a chemical curiosity, even at a 0.01-percent impregnation imparted resistance to attack for 136 days and had a regular curve to 259 days at 0.5, and 303 days at 1 percent, which was the greatest concentration at which the minute sample could be impregnated.

The chemicals which at 0.5-percent concentration of impregnation protected samples from termite attack for 11 days (or less) were: Dibromonaphthalene, 2-chloro-6-nitrotoluene (duPont), chloramine T (chlorazone 1,4), di-(2,4-dichlorphenyl) carbonate (Laurel Hill), rufigallol, 2,4,6-tribromophenol, phenyl salicylate, 4,4′-dichlorbenzophenone (Dow), polymerized methyl methacrylate, 4,4′-dibromodiphenyl, and L-rhamnose hydrate; for 13 days: Laurel Hill experimental chemicals such as a condensation product of potassium acetaldehyde disulfonate and T-butyl-phenol and the monochlor of the same, 4-chloro (2-phenoxy) benzoate, hexachloracetone, phenol potassium acetaldehyde disulfonate, a distilled neutral 50-percent fraction of cardol supplied by General Food Laboratories, sodium tetrahydronaphthalene-β-sulfonate, oxine,8-hydroxyquinoline, resorcinol monomethyl ether, hematoxylin, and diphenyl triketone. The only one of these chemicals notably more resistant to termite attack at greater concentrations was diphenyl triketone, which at 2 percent was not finally eaten until after 497 days.

To the uninitiated it would seem that if a phenolic compound were termite-repellent, a diphenol would be twice as repellent. Tests disproved this. Wood samples submerged for 10 minutes in 0.5-percent diphenylene oxide were eaten in 3 days; in 0.5-percent diphenyl in 6 days. The clear, sparking series of diphenyls, called "Aroclor" by Monsanto, proved to be

very much of a disappointment, for the termites ate the treated samples so greedily as to leave no doubt. The flamboyán block submerged for 10 minutes in 0.5 percent of tetrachlordiphenyl was eaten in 10 days; of pentachlordiphenyl in 12 days; of hexachlordiphenyl in 21 days; of its trisulfide in 8 days; of heptachlordiphenyl in 12 days; of decachlordiphenyl in 8 days; of nonachlorterphenyl in 19 days. No blocks impregnated with greater concentrations proved to be much more resistant.

The 2-amino-xanthone and 2,7-diamino-xanthone from Laurel Hill proved to impart less resistance to termite attack than xanthone itself, the samples impregnated with 0.5 percent being eaten in 15 days. The mosquito- and punkie-repellent chemicals manufactured by Dodge & Olcott: Caryophylene and "Dimelone" (dicyclo-(2,21)-5-heptene-2,3-dicarboxylic acid, dimethyl-ether), lacked permanence under exposure to the air, and the impregnations on wood samples at 0.5 percent were eaten by termites after 16 to 17 days, greater concentrations being little more effective. A heavy chlorination of crude anacardic acid prepared by General Foods proved no more permanently repellent. The β-chlor-anthraquinone impregnation at 0.5 percent was attacked in 16 days, but the 2 percent resisted for 178.

The following chemicals in impregnations at 0.5 percent resisted termite attack for not more than 15 to 18 days: Saponin, a wood constituent supplied by S. B. Penick & Co.; 2,4-dichlor-phenoxy-acetic acid (duPont); 2-chlor-orthophenyl-phenol (Dow); carbostyril, or 2-hydroxy-quinoline; nickel pentachlorphenate, contributed by International Nickel Co.; Lederle's sulfanilamide (*p*-amino-benzene-sulfonyl-amide), zinc lake (OH) of quinizarin, *p*,*p*-difluorodiphenyl, sodium tri-iso-propylnaphthalenesulfonate. An 18-percent chlorinated crude cashew nut-shell oil synthesized by General Foods, and guaiacol, presumably extracted from the heartwood of lignum-vitae, *Guaiacum officinale* L., but obviously not the constituent responsible for its complete resistance to termite attack, gave protection for 21 days.

A 0.5-percent solution of Lederle's sulfathiozole protected wood only 23 days against termite attack, as did quinone, pentachloracetone, triphenyl phosphine, quinhydrone, and Dodge & Olcott's phenol B.; a 0.5-percent impregnation of Naugatuck's Chloranil (tetrachlor-para-benzoquinone) protected wood 25 days, and a 2-percent only twice as long. Two coal-tar constituents submitted by Reilly were much more effective at greater concentrations, pyrene at 2 percent imparting resistance for 195 days and fluoranthene for almost a year, but phenanthrene (Koppers) only 87 days.

The Osage orange crystals manufactured by the Taylor, White Extracting Co. of Camden, N. J., apparently contain very little of whatever con-

stituent of Osage orange wood is responsible for its resistance to termite attack, for impregnations with a 0.5-percent solution prevented termite attack for only 27 days, and with 1 percent for 33. Quinalizarin and the zinc lake (OH) of anthrarufin and chrysazin showed no greater promise, nor did 3,5-dinitrosalcylic acid or 2,4-dihydroxydiphenyl, sodium 2,6-dibromobenzononeindo-3-bromophenol, pentachlornitrobenzene (Laurel Hill), or the copper lake of Eastman's dihydroxyanthraquinone.

Copper naphthenate, extensively used for the protection of the bottoms of wooden ships and boats against the attack of marine worms, is of little value against dry-wood termites. It is a very dark-green, foul-smelling, sticky liquid, but impregnation with a 0.5-percent solution prevented termites eating the samples only 28 days. Although the 1-percent impregnation held out for 150 days, only the 10-percent gave complete protection. If it seems incredible that the termites minded it so little, it must be remembered that their senses of taste and smell are quite different from ours, and what seems extremely offensive to us may not produce the same reaction in them.

The commercially unobtainable catechin, supposedly a decay- and insect-repelling wood constituent, was eventually received from General Foods as *l*-epicathechin, but the 0.5-percent impregnation resisted termite attack only 29 days, and the 1-percent, 31 days. Their sample of anacardic acid was also disappointing. Similarly valueless were bis-(1,2,3-trichlorphenyl) methane and perchlorhydrindene from Laurel Hill, benzophenone, triphenyl phosphite, and the wood constituents thanite (Hercules Powder), linalool, and brucine. Because glossy paper coated with aluminum sulfate is discriminated against by termites when uncoated paper is available, high hopes and great expectations attended the tests with aluminum pentachlorphenate, supplied by Dow, but it was no better than dicyclohexamine pentachlorphenate, neither being even measurably comparable in repelling termites to pentachlorphenol itself.

Termites ate the flamboyán wood impregnated with 0.5-percent anthragallol in 33 days, and that with 2-percent in 66. The Eastman preparation of saponin gave results closely paralleling those with saponin from another source, and impregnations with their 3-bromo-ace-napthene was also eaten at 0.5 percent in 33 days. Before the chemists had learned how to synthesize pentachlorphenol, tetrachlorphenol was the most heavily chlorinated phenol available. Its inferiority was shown when flamboyán was impregnated with a 0.5-percent solution of the sample supplied by Dow, and was eaten in 37 days, at 1 percent in 46 days, and at 2 percent in 116 days.

When DDT was just beginning to attract attention as used by the U. S. Army, a minute sample was obtained through the courtesy of F. C. Craighead of the Forest Insects Division of the then Bureau of Entomology and

Plant Quarantine, USDA. The sample of flamboyán wood impregnated with 0.5-percent DDT was eaten 35 days after submersion and with 1-percent in 38 days; but still uneaten over 10 years after the date when it was dipped in the chemical is the one impregnated with the 2-percent DDT. When termites do not like a sample, they often deposit liquid excrement on it; the 2-percent sample is almost covered with the spots, showing how little it was appreciated by them. In considering the use of DDT for commercial impregnation it should be noted that it is only half as repellent as pentachlorphenol, and that considerably more than a 2-percent solution should be used to give an ample margin of error.

Triphenyl stibine may be as effective as DDT, samples impregnated with its 0.5-percent solution not having been eaten for 45 days and the 1-percent for nearly 2 years (686 days). But no impregnation at 2 percent was prepared, and it remains untested at this concentration. The zinc lake (acetic acid) of anthragallol at 0.5 percent repelled attack for 37 days, and at 1 percent for 90; the copper lake of anthragallol repelled attack for 44 and 134 days, but neither was tested at greater concentrations.

Indeed, it is really rather pointless to carry tests beyond the concentration at which pentachlorphenol imparts resistance, and also many chemicals are little more repellent at the greater concentrations. For instance, the impregnation of anthraquinone at 0.5 percent was eaten in 43 days, the 1 percent was eaten on the very next day, and the 2 percent 2 days later. Samples impregnated with 0.5-percent solutions of two experimental chemicals submitted by the Laurel Hill Laboratories, hexachlorcyclopentadiene dicyclopentadiene and a highly chlorinated adduct of monochlor potassium acetaldehyde disulfonate with phenol, were eaten in 39 and 43 days, respectively, and were only somewhat more resistant when impregnated with greater concentrations. Pentachlorphenyl laurate submitted by Monsanto proved slightly more repellent, but its 2-percent-solution impregnation was attacked in 128 days. A mixture of 65 percent of 1,5-dihydroxyanthrarufin and 35 percent of 1,8-dihydroxychrysazin (Eastman), when dissolved in lutidine and used in 0.5-percent solution to impregnate flamboyán wood prevented attack for 48 days, but dissolved in sodium hydroxide, the 0.5-percent impregnation was eaten in 4 days, and the 1-percent in 10.

When chlordane was so new that only a small experimental sample could be furnished by Dow, the 0.01-percent dilution made the wood sample initially toxic and killed all the termites in the test, but after a 2-week exposure in the air it was no longer toxic, and the samples were eaten at increasingly greater concentrations up to 0.5 percent in 48 days. The 1-percent-solution sample was not attacked for over a year, and the 2-percent impregnation proved repellent for 378 days. The sample of 4-chlor-

benzophenone submitted by Dow was not toxic at all and blocks on which it was used were promptly attacked at the lower concentrations, but were exactly as resistant at 0.5 percent as if impregnated with DDT, and less so at 1 and 2 percents.

Isothymoxy chloroethyl ether, the solvent C of Dodge & Olcott, proved to be more repellent to termites than most chemicals that it might be used to dissolve, the impregnation at 0.5 percent not being attacked in 53 days, and at 2 percent for twice as long.

The following chemicals at 0.5-percent-solution impregnations made flamboyán wood samples resistant to termite attack for over 50 days, but were little more repellent at greater concentrations: Phenanthrene quinhydrone, hexaethyl tetraphosphate (Monsanto), quinizarin or 1,4-dihydroxyanthraquinone, a Penn Salt glycol (alkyd) resin of 2,3,4,5-tetrachlorphthalic anhydride, the copper lake of alizarin, and the least repellent two of a series of benzalaniline compounds synthesized in the organic chemistry laboratories of the Royal Institute of Technology, Stockholm, Sweden, and sent by Erdtman: 3,4-dimethoxybenzalaniline and 2,3,4-trihydroxy-benzalaniline.

A 0.5-percent solution of the dinitro-o-phenyl phenol submitted by Dow gave immunity to termite attack to the sample of flamboyán wood impregnated for 69 days; the 1-percent sample is still unattacked. On the basis of our tests, and insofar as the West Indian dry-wood termite is concerned, this chemical is as good as pentachlorphenol and has the additional advantage (or disadvantage) of deeply staining the light-colored wood sample an intense yellow, so that one may be assured that the wood has been adequately covered on every visible part of its surface. Not so attractive in appearance is the Dow experimental textile fungicide, K-4639, a cupric salt of 3-phenylsalcylic acid, which when dissolved in benzol is a densely dark-brown solution lightly staining light-colored woods. Impregnation with 0.5-percent solutions protected flamboyán wood for 79 days; the 1-percent samples had not been eaten 6 years later. On the basis of our tests it too may be considered as practically equivalent to pentachlorphenol in protecting against the attack of the West Indian dry-wood termite.

Many other chemicals which repel termite attack at impregnations of 0.5 percent provide little more resistance at 1 percent and greater concentrations. The Naugatuck experimental chemicals 2,3-dichlor-1,4-naphtoquinone and 2,3-dichlor-naphthoquinone with sodium isothiocyanate at 0.5 percent provided protection for only 74 to 78 days, and at 2 percent slightly less than twice as long. Samples impregnated with Laurel Hill's 2,2-di(4-hydroxy-3,5-dichlorphenol) propane, dichlorxanthone, and chloral 4-chloraniline at 0.5 percent were eaten in 71, 75, and 79 days, respectively.

At greater concentrations the chemicals repelled attack for not much longer periods.

Impregnations with 0.5-percent solutions of chrysin (5,7-dihydroxyflavone), a constituent of *Pinus lambertiana* Douglas, extracted in Erdtman's laboratories in Sweden, repelled attack by *Cryptotermes brevis* for 55 days, but in less than twice as long a period samples impregnated with it at 1 percent were eaten. Alizarin (1,2-dihydroxy-anthraquinone) similarly imparted resistance for months at low concentrations, but in less than 4 months the deeply stained 2-percent impregnations were attacked, the termites on the day following such a meal showing the purplish color of their food right through the thin walls of their transparent abdomens.

Solutions of osajin, extracted from Osage orange, *Maclura pomifera*, by M. L. Wolfrom of Ohio State University, and named and its chemical structure established by him, when used for impregnating flamboyán wood at such an extreme dilution as 0.002 percent repelled termite attack for 39 days. But at 0.2 percent the sample was attacked in 61 days, and presumably osajin is little more repellent at the greater strengths which could not be tested because of the small sample submitted. A smaller sample of iso-osajin monomethyl ether proved to be even more repellent, repelling attack for 47 days at 0.002 percent, but at 0.1 percent the sample was eaten in 73 days. It is doubtful whether either it or osajin is the important constituent of Osage orange wood responsible for its termite-resistance rating of 90.

THE MORE EFFECTIVE CHEMICALS

Of the experimental chemicals submitted by the Laurel Hill laboratories, impregnations of a 0.5-percent solution of di-(4-hydroxyphenyl)2,2,2-trichlorethane imparted immunity to termite attack for 82 days, and for a little more than three times as long at 2 percent. Dibromoxanthone at 0.5 percent repelled attack for 83 days; trichlorethyl-trichlorvinyl sulfonate for 91, and chloranil cyclopentadiene for 95 days. Laurel Hill's 4-iodox xanthane, of which the 0.5-percent solution conferred immunity to termite attack for 124 days, has not yet been eaten at 1 percent, 6 years after impregnation. This is also true of their 2,4-dichlorphenyl-2-phenoxy benzoate, but unfortunately we had no sample of their 2-ethylhexyl-5-nitrosalicylate, attacked by termites at a 0.5-percent impregnation, for test at a greater concentration. Hexachlorcyclopentadiene-2,4-dichlor-phenyl-allyl repelled attack at 0.5 percent for 133 days; a benzoquinone reaction product with hexachlorcyclopentadiene prevented attack at 0.5 percent for 147 days; benzoyl xanthone prevented attack for 148 days; and penta-

methyl flavan for 157 days; but these also were not tested at greater concentrations.

The zinc lakes (acetic acid) of quinizarin and of β-chlor-anthraquinone were used to impregnate flamboyán wood samples in 0.5-percent solution, and both were attacked in 96 days, with the 1-percent impregnations immune to termite attack 8 years after treatment. A comparable similarity was shown by 3,4-methylene dioxystilbene and 3,4-dimethoxystilbene, experimental chemicals sent by Erdtman, of which the termites attacked the impregnations at 0.01 percent in 63 days for both chemicals; in 86 or 88 days at 0.5 percent, but took twice as long at 1 percent. No such detailed data are available on two other wood extractives sent by Erdtman, cedrol and the glucoside rhapontin for, attacked at the lowest concentrations after a few days, the 1-percent samples when left in termite-infested boxes for 15 months were found eaten when finally examined. A longer period of neglect for the 0.5-percent and 1-percent impregnations of α-naphthoflavone, uneaten by the termites for a number of years and finally left undisturbed in a termite-infested display cabinet, is responsible for the lack of exact data concerning the period of something less than 9 years during which they continued resistant. Eventually deep tunnels were noted in the back. Pinocembrin (5,7-dihydroxy-flavonone), extracted from *Pinus lambertiana* Douglas in Erdtman's laboratory, was not nearly so repellent to termite attack, its 0.5-percent-solution impregnation being eaten in 107 days and the 1-percent in 288.

Tests with the very toxic sodium fluoracetate, Monsanto's "1080", resulted in the death of many termites. Nevertheless, they ate the samples impregnated with 0.5-percent solution in 107 days and with 1-percent in 167. This chemical is much too dangerous to use for wood impregnation since many comparatively innocuous chemicals are available which are merely repellent, and not toxic, and which are much better for this specific purpose.

The 3,5-dinitro-o-cresol impregnation with 0.5-percent solution protected wood from attack 96 days, and at 2 percent three times as long. Dinitro-sec-amylphenol imparted more permanent resistance, the 2-percent-solution impregnation not being attacked for 535 days. Ammonium-dinitro-ortho-sec-butyl phenol, of which "Sintox W" of the Standard Agricultural Chemicals Co., Hoboken, N. J., contains 12 percent, which was the source of the material for testing, impregnated with 0.2-percent solution repelled termite attack for 59 days, just the same length of time that the 3,5-dinitro-o-cresol repelled attack, but its 0.5-percent impregnation was not attacked by the termites in 6 years. At this dilution the chemical stains the wood treated a bright yellow, positive proof that its protective film covers all surfaces of the submerged lumber. This material is com-

mercially available in quantity, and on the basis of these tests, is preferable to dinitro-o-phenyl phenol, the other bright-yellow staining dinitro compound. A 0.5-percent solution of Dow's 4,6-dinitro-o-sec-butylphenol prevented attack for 244 days; at 1 percent the sample has not been eaten in 7 years.

Blocks impregnated with a 0.5-percent solution of isobornyl thiocyanoacetate, contributed by the Hercules Powder Co., resisted termite attack for 132 days, but the 1-percent impregnation was eaten in less than twice that time. Dibenzyl, of which the impregnation at 0.5 percent was eaten in 121 days, was not tested at greater concentration because of the small sample sent by Erdtman. Physodic acid, available in greater quantity up to a 1-percent impregnation, survived in the tests for 162 days. Diphenyl octatetraene, also supplied by Erdtman, but for which no solvent was found at more than 0.1-percent concentration, prevented termite attack for 123 days on wood impregnated with the saturated solution.

Piperonyl butoxide, the triumph of Dodge & Olcott in their researches with pyrethrum, gave an immunity to termite attack in flamboyán wood submerged in a 0.5-percent solution for 181 days, in 1-percent for 229, and in 2-percent for 287. Samples impregnated with their piperonyl cyclohexenone at a 1-percent dilution were uneaten after over 7 years. Penn Salt's experimental chemical 2-ethylhexyl-2,3,4,5-tetrachloracetate, when used for impregnating flamboyán wood at 0.5-percent concentration, protected it from termite attack for 190 days. Obviously it was not tested at sufficient concentration to indicate whether it was in reality better than Laurel Hill's genicide acids, of which the 0.5-percent impregnation was attacked in 163 days, but the 1-percent is still uneaten after nearly 7 years. Laurel Hill's pentachlorphenyl diethyl thionphosphate impregnation, eaten at 0.5 percent and 1 percent, remained untouched at a 2-percent concentration after 5 years. A sample inpregnated with diphenyl mercury at 1-percent concentration is still sound after nearly 9 years, and so is one impregnated with phenylmercuric chloride at 2-percent.

Impregnation with a 0.5-percent solution of 3,4-methylenedioxybenzalaniline prevented termite attack on flamboyán wood for 204 days. This statement, independent of any explanation, seems rather pointless. Actually, this chemical is only one of a series of benzalaniline compounds prepared in the organic chemistry laboratories of Holger Erdtman with the idea that, on the basis of chemical analogy, some might prove more permanently repellent to termite attack than the wood extractives, pinosylvin, and stilbene. A preliminary and incomplete account of these results has already been published (4); this is a more complete summary.

Benzalaniline itself, in which nitrogen replaces one of the ethylene bonds of diphenylethylene or stilbene, is not as repellent to termites as is stilbene,

but the 0.5-percent-solution impregnation on flamboyán wood prevented attack for 318 days. Some of the compounds formed with it were much less repellent; the 3,4-dimethoxy at 0.5-percent giving protection against termite attack for only 52 days, and 2,3,4-trihydroxy for 53. As stated at the beginning of this paragraph, the next most repellent in the series is 3,4-methylenedioxy, for 204 days. The 2-hydroxy at 0.5 percent conferred immunity for 267 days; 3-methoxy-4-hydroxy for 272; 2-nitro for 294; 3-methoxy-4-hydroxy-5-chloro for 316; the 4-methoxy for 341; the 4-hydroxy for 357; the 4-chloro for 417; the 2-hydroxy-3-methoxy for 873; the 3,4-dihydroxy for 929; the 2,4-dichlor for 952 (or over 2½ years) but the 2,4-hydroxy permitted attack at 0.002-percent only after 257 days, and at 0.005 percent still produces immunity nearly 3 years after impregnation. Under constant test the sample shows no sign of being even attractive for the termites to rest on.

Azobenzene, in which nitrogen completely replaces the double ethylene bond of diphenylethylene, even at the greatest dilutions proved to be more repellent than benzalaniline, and after the termites had attacked the wood sample impregnated with a 0.02-percent solution in 142 days, they showed no interest in the 0.05-percent impregnation made nearly 3 years ago. Both benzalaniline and azobenzene are commercially available, and numerous experimental compounds with the former, besides those sent by Erdtman, have been tested against mites, ticks, and insect pests attacking man (1), but only one with azobenzene. By analogy, a 2,4-dihydroxyazobenzene would seem to be indicated as the ultimate in termite-resistance. The sample synthesized by the chemists of Nuodex International, courtesy W. J. Strossen, is bright crimson, staining flamboyán wood orange, and at lesser strengths, yellow. With tests just started, it proves to be not nearly as repellent as azobenzene, impregnations at 0.5 percent being eaten in 49 days. An impregnation in flamboyán wood with a 0.05-percent solution of the sodium salt of 2,5-dichloro-4′-hydroxy-azobenzene (Penn Salt) protected it less than a month, but the 0.1-percent impregnation remained untouched a year later. With the greatest difficulty, 2,4-dihydroxyphenanthrene (a dehydro cis-pinosylvin) was prepared in Erdtman's laboratory, and a wood sample with a 0.05-percent impregnation resisted attack for 594 days. Phenanthrene itself at an impregnation of 1-percent was attacked in 8 days.

Because a suitable solvent for salazinic acid was not found, the greatest concentration at which the wood sample could be impregnated was 0.1 percent. Initially very repellent at the greatest dilutions, a 0.002-percent film being resistant to the termites for 109 days, the 0.1-percent saturated-solution impregnation was eaten in 126 days. Vulpinic or lichen acid at 0.1-percent impregnation resisted attack for 159 days, and the 0.2-percent remained immune to attack 3 years after treatment. Equally resistant at

the greater dilutions was *d,l*-usnic acid, but the 0.5-percent-solution impregnation was eaten in 312 days, and the 1-percent in 467. Erdtman had sent these accompanying other wood extractives. The sample impregnated with 0.5-percent 1,5-dibromo-pinosylvindimethyl ether was attacked in 213 days, and with the 1-percent in 403 days. Though the 0.5-percent impregnation proved to be resistant for over a year, the sample of 2,3,6,7-tetramethoxy-9-10-dihydrophenanthrene was too small for test at greater concentrations.

The major insect- and fungi-repellent constituent of Scotch pine, *Pinus sylvestris* L., supplied for these tests by Holger Erdtman of the Royal Institute of Technology at Stockholm, Sweden, was initially remarkably resistant to termite attack, flamboyán wood impregnated with pinosylvin at 0.01 percent remaining immune for 565 days in our tests. But subsequently, at greater concentrations, the impregnations proved less permanent, so that at 0.5 percent the wood sample was eaten in 670, and at 1 percent in 674 days. Pinosylvin is 3,5-dihydroxystilbene, and 0.5 percent of stilbene itself, a commercially available chemical, rendered the impregnated wood sample repellent for 826 days, and at 1 percent for 893 days, or somewhat more than 2 years. Other wood constituents, or some compounds synthesized from pinosylvin, are even more permanently repellent. The dihydropinosylvin monomethyl ether, of which the 0.5-percent impregnation resisted attack for 1,278 days, at 1 percent produced immunity for over 5 years. Pinosylvin dimethyl ether, of which the 0.2-percent impregnation was eaten in 577 days, is unattacked at 0.5 percent 3 years after impregnation. By far the most repellent of these wood constituents was pinosylvin monomethyl ether, of which the flamboyán sample impregnated at 0.01 percent remained untouched nearly 6 years later. Indeed, it is almost covered by the spots of dried liquid excrement with which the dry-wood termites eventually adorn a bit of wood unamenable to their voracity.

The flamboyán wood sample impregnated with 1 percent of 2,4,6-trinitrostilbene resisted attack for almost 4 years, and the 0.2-percent impregnation of 2,4,2′,4′-tetranitrostilbene ceased to be immune after 2 years and 4 months. Wood impregnated with 0.1-percent solution of 2,4-dichlorstilbene was not eaten for 277 days, and the 0.2-percent is still uneaten. Most repellent of all are the 2,4-dinitro-2′,4′-dichlor-stilbene, and 4,4′-dinitro-stilbene; the wooden samples impregnated with dilutions of 0.005 percent were uneaten after 4½ years.

The wood of the Australian cypress pine, *Callitris glauca* R. Brown, has such a horrible, penetrating, and persistent odor that even termites are repelled by it, and the wood has been given a termite-resistance rating of 100+. No sample of callitrol was available to Erdtman, but he was able

to obtain (plus)-citronellidene acetic acid which is a synthetic analogue of (plus)-citronellic acid. The termites could scarcely distinguish between the two, impregnations with a 1-percent solution being attacked in 561 to 569 days. This still does not indicate whether the minus extractive would be as comparably powerful in repelling termites as is the wood from which it is obtained.

The wood of Douglas fir, *Pseudotsuga taxifolia* (Poir.) Britton, is very susceptible to attack by *Cryptotermes brevis*, especially when available as plywood, and its heartwood has a termite-resistant rating of 55, its sapwood of 49. It is possible, however, that the extractive taxifolin (3,5,7, 3′,4′-pentahydroxoflavonone) is present in very small amount, for impregnation on flamboyán wood of a 0.02-percent solution repelled attack for 154 days, and the sample treated with 0.05-percent was not attacked in nearly 4 years.

From the sample of chlorphorin sent to Erdtman, extracted by F. E. King of Nottingham University, England, from *Chlorophora excelsa* Benth. & Hook f., an African tree commercially called iroko, a fraction was forwarded for tests with *Cryptotermes brevis*. When used to submerge flamboyán wood, the 0.2-percent-solution sample resisted attack for 234 days, and the 0.5-percent block remained uneaten over 3 years later.

Tectoquinone (β-methylanthraquinone) from East Indian teak, *Tectona grandis* L., has the best record, for the length of time during which the impregnated wood has resisted attack, the 0.5-percent-solution impregnation having been attacked after 536 days, but the 1-percent, despite repeated tests, remained unharmed 9 years after impregnation.

Most of the extractives which M. L. Wolfrom obtained from Osage orange, *Maclura pomifera* (Raf.) Schneider, were available in such small quantity that one could but report that they were not very effective in preventing termite attack at the greater dilutions. The principal one, pomiferin, could be supplied in sufficient amount so that a 1-percent solution was tested. The 0.5-percent-solution impregnation on flamboyán was eaten in a little over a year, but the 1-percent continued to resist termite attack for 880 days, or approximatedly 2½ years. One other extractive which Wolfrom calls "substance I," for which no structural formula has been proposed, but of which the molecular formula is $C_{23}H_{24}O_6$, is much more resistant to termite attack than is pomiferin. It is bright yellow in color and stains flamboyán so intensely that termites eating the 0.05-percent-solution impregnation after 236 days had abdomens bright with the purplish comminuted wood particles in process of digestion. When impregnated at greater concentrations the termites avoided the samples for over 2 years, and "substance I" may be more largely responsible for Osage orange being resistant to termite attack than the more obvious

pomiferin. Of the other extractives, pomiferin trimethyl ether at a 0.2-percent wood impregnation was not eaten for 112 days; isopomiferin dimethyl ether at 0.1-percent resisted attack for 225; tetra-hydro-osajin at 0.2-percent prevented termite attack for 707, but in every case the sample submitted by Wolfrom was so small that these data represent the end of the test with that particular extractive and give no realistic picture of what might be shown at the greater concentrations.

Some of the more repellent to termite attack of the chemicals submitted by the Laurel Hill laboratories were parachlorometa xylenol, flamboyán samples impregnated with 0.5-percent solution finally being attacked 277 days later; α-phenyl-4-hydroxy-cinnamic acid after 319; resorcinol acetone after 354; chlorinated phenol potassium acetaldehyde sulfonate after 319 days, and when the 2-percent solution was used, in just a year; 2,4-chloro-phenol monochlor potassium acetaldehyde disulfonate after 523 days; the 1-percent in 561 days, and the 2-percent after 2½ years; butyl 4-chloro-benzoyl acrylate at 0.5-percent after 661 days; ortho-dichloro-benzene-fumaryl-chloride, of which the 0.2-percent impregnation was eaten in 47 days, but the 0.5-percent impregnation was not eaten in over 2½ years; hexachlorcyclopentadiene dimer, of which the 0.2-percent impregnation was eaten in 166 days, but the 0.5-percent sample remained uneaten after 5 years; dibenzoyl ethylene, of which the 0.2-percent resisted attack for 341 days, and the 0.5-percent impregnation was not eaten 2½ years later; phenol heptachlor-propane, of which the 0.02-percent impregnation resisted attack for 667 days, the 0.2-percent for 675, and the 0.5-percent was not attacked in a little over 2 years; oxygenated hexachlor-cyclopentadiene dimer, of which the 0.2-percent impregnation resisted attack for 675 days, and the 0.5-percent was not attacked in 5 years; quinone-cycloactatetraene which imparted resistance for over 2 years at a 0.05-percent impregnation, but at 0.5-percent was eaten in 988 days.

Xanthone (Laurel Hill) was but slightly repellent to termite attack in diluted applications, the sample treated with 0.2 percent being eaten in 67 days, but at 0.5 percent the treated flamboyán wood resisted attack for 1,053 days, and the 1-percent sample was not attacked after nearly 7 years. In the hope that some combinations of xanthone might be even more resistant, a tetrachlorxanthone was something of a disappointment, with the 0.5-percent-solution impregnation eaten in 468 days, the 1-percent in 592 and the 2-percent in 594. But the 2-methoxy and the 4-methoxy at a 0.2-percent concentration prevented attack for 182 to 200 days, and the 0.5-percent impregnations were not eaten after 6 years.

Of the variants of the basic DDT formula, Laurel Hill submitted 1, 1-diphenyl (4-hydroxy-3,5-dichlor) 2,2,2-trichlorethane, of which the 0.5-percent impregnation prevented attack for 454 days and the 1-percent

for 514. The 1,1-di(4-hydroxy-2,5-dichlorphenyl) 2,2,2-trichlorethane at 0.5 percent was not eaten for 615 days, and the 1-percent impregnation remained uneaten 5 years afterward. As an extreme indication of how long a test must be conducted before one can be at all sure of the result, 1,1-di(2-hydroxy-3,5-dichlorphenyl) 2,2,2-trichlorethane is an outstanding example, for the impregnations using 0.05-percent solutions were eventually eaten over 5 years after impregnation, and the 0.1 percent only 3 days later, with the results on exposure of the 0.2 percent to termites as yet uncertain, presumably awaiting another 5 years of test. Thus one must consider with caution 1,1-di(4-hydroxy-2-methyl-5-isopropyl) 2,2,2-trichlor-ethane, of which the 0.05-percent impregnation was eaten in 38 days, but the 0.1-percent resisted attack almost 2 years later; and 1,1-di(4-hydroxy-2-methyl-5-chlorophenyl) 2,2,2-trichlorethane, of which samples impregnated with the 0.005-percent solution was eaten in 19 days, at a 0.01-percent impregnation remained resistant for over 1½ years.

The Q-137 of Rohm & Hass, 1,1-bis (*p*-ethylphenyl)-2,2-dichlorethane, impregnated on flamboyán wood at 0.5 percent prevented attack for over 2 years, but has been available for test for such a short additional time that no prediction can be made with any assurance as to its permanence at greater concentrations. The same is true of Prentox Strobane, originally produced by Prentiss Drug & Chemical Co., now transferred to B. F. Goodrich Chemical Co., of which the 0.02-percent impregnation prevented termite attack for 711 days.

Diazinon, originally synthesized by H. Gysin in the laboratories of J. R. Geigy S. A., Basle, Switzerland, with the formula O,O-diethyl-O-(2-isopropyl-4-methyl-pyrimidyl-6) thionphosphate, was made experimentally available in the United States by the Geigy Co. Inc., late in 1952. The flamboyán wooden block impregnated with a 0.1-percent solution was attacked just 2 years after treatment. It is too early to judge what its eventual value may be.

UNDESIRABILITY OF TOXICITY

The fundamental distinction between ordinary insecticides and chemicals being tested for permanence in immunizing wood to termite attack is that the former are toxic and the latter preferably nontoxic, for toxicity to termites of any chemical will presumably make it temporary in its repellent properties. Thus the metallic dimethyl-dithiocarbamates produced by du Pont primarily as fungicides, but which, incidentally, are toxic to termites at great dilutions when impregnated on wood samples, eventually loose this toxicity and thus demonstrate that their repellent property is temporary. In less than 2 years after impregnation at 0.1 percent both the copper and zinc compounds were no longer toxic on samples of flamboyán

wood, and no longer protected it against attack. Ferric dimethyl-dithiocarbamate, or "Fermate", was also originally toxic, but 3 years after impregnation the wood samples impregnated with a 0.5-percent solution were eaten. The *p*-chlorphenyl *p*-chlorobenzene sulfonate of Dow is also initially toxic, but a year after impregnation at 0.05-percent the wood sample was attacked, and in 455 days even the 1-percent sample was eaten. Four years elapsed before the sample impregnated at 2 percent could be attacked by the termites, and even then the form of attack was deep tunnels into the interior of the sample, rather than superficial gouges on its face.

When the chemists of the Hercules Powder Co. were perfecting the insecticide "Toxaphene", one of the experimental samples submitted for test with termites was a crude 60-percent chlorinated terpene which was initially toxic, but of which impregnations at the 0.01-percent level could be attacked with impunity by the termites in 71, the 0.1-percent in 296, and the 1-percent in 484 days. This should not be interpreted as being equivalent to the residual effect of toxaphene applied as an insecticide, for all the applications of chemicals in the tests with termites were of submersion 10 minutes in a solution, which is quite different from superficial spraying of an emulsion, or a suspension in water.

Wood of the Trinidad and South American shrub, *Ryania speciosa* Vahl., placed in test with other woods in a petri dish, not only repelled the termites but within a few days killed all of them, even though they ate none of the wood. Its extractive, Ryania or Ryanodine, originally developed by Merck and later taken over by S. B. Penick & Co., and made commercially available as an insecticide, is correspondingly powerful. The chemists have not yet determined the chemical composition of Ryanodine. The sample of the undiluted commercial preparation when used at a 0.02-percent level to impregnant flamboyán was eventually attacked in 204 days, although at first it has been too toxic for test. The wood sample impregnated with 0.05 percent of Ryania was eaten slightly at a later period, but remained substantially repellent 5 years after impregnation, one noteworthy example of a chemical initially toxic which continued to be repellent.

The sample impregnated with commercially available and widely used water-soluble sodium pentachlorphenate (Monsanto), at the dilution at which Ryania resisted attack for 5 years at the 0.05-percent level, was eaten in 38 days, but at 10 times this concentration (0.5-percent) it withstood attack for nearly 10 years. Impregnation of wood with zinc pentachlorphenate at the 0.5-percent level was eaten in 650 days, and no sample with a greater concentration had been prepared for test. Actually none seems required, for copper pentachlorphenate, of which the impregnation at the 0.1-percent level was attacked in 111 days, withstood termites at 0.2 percent for over 9 years. In our experience no difficulty has been experienced in obtaining

perfect solutions at tropical temperatures, but it must be admitted that, although articles impregnated with copper pentachlorphenate are not stained and give no apparent indication of having been treated, a sprayed basket in which green bananas are brought from the market bears numerous purplish-brown spots where the juice oozed from the freshly cut stems.

Among the chemicals which enabled wood to withstand termite attack for long periods, triphenyl phosphate at a 0.5-percent level conferred protection on the flamboyán sample for 533 days; 3,4,3′,4′-tetramethoxydibenzyl at 1 percent for 4 years; the zinc lake (acetic acid) of alizarin at 0.5 percent for 512 days; and the zinc lake (acetic acid) of anthrarufin and chrysazin, attacked at 0.2 percent in 96 days, resisted attack at 0.5 percent for 8 years. The adduct of abietic acid and maleic anhydride which Erdtman thought might show promise, has been under test only 2 years, but at the end of this period the 0.5-percent impregnation had been eaten by the termites.

Because the 0.1-percent impregnation of dimethyl-tetrachlor-phthalate of the Whitemarsh Research Laboratories of Penn Salt had resisted termite attack for nearly 3 years, it seemed possible that other combinations might be even more valuable. As it happened, however, the 0.1-percent concentration was eaten in 1,226 days, the 0.2-percent in 1,228, and the 0.5-percent was attacked 5 years after the original impregnation. Thus it is hardly surprising that the *n*-propyl of the 2,3,4,5-tetrachlorphthalate at a 0.5-percent level of impregnation was eaten in 623 days; a glycol (alkyd) resin at 0.5 percent in 643; a glycerol (alkyd) resin at 0.5 percent in 645; and a di-2-ethylhexyl at 0.2 percent in 659 days. The 0.5-percent-impregnated wood has not yet been attacked after a little more than 2 years in the test. The sample impregnated with 2,3,4,5-tetrachlor benzoic acid at a 0.02-percent level was eaten in 54 days, but there is as yet no report on the 0.05-percent dilution.

A miticide of Naugatuck Chemical Co., called "Aramite," imparted resistance to termite attack to wood for over 4½ years when used at a 0.5-percent concentration, but we have no report on the 1-percent impregnation.

Mallinckrodt's pyridyl mercuric stearate showed a remarkably even resistance to termite attack: The impregnation at a 0.1-percent level was attacked in 19; the 0.2-percent in 440; the 0.5-percent in 1,241 days; the 1-percent impregnation remained uneaten for 9 years. In all the other compounds of mercury this element has been only a unstable point of weakness, but Mallinckrodt's pyridyl mercuric chloride protected the wood sample when impregnated with a 0.2-percent solution for 9 years before it was attacked.

Because Commercial Solvents' "Dilan" has not been commercially or

experimentally available for so long a time, one can only report that the propane fraction of 2-nitro-1,1-bis (*p*-chlorphenyl), at a 0.5-percent level of impregnation protected the sample of flamboyán wood, for 4½ years, and at 0.2-percent the butane fraction did the same.

The first sample furnished by du Pont of their methoxychlor, 2,2-bis (*p*-methoxyphenyl) 1,1,1-trichlorethane, late in 1949, is still an effective preventive of termite attack at an impregnation of the 0.05-percent concentration on flamboyán wood. A subsequent sample consisting of 90 percent of (*p*,*p*-methoxyphenyl) and 10 percent of (*o*-methoxyphenyl), proved less effective, the 0.1-percent impregnation being attacked in 351 days.

Five years ago Nuodex Products submitted a sample of their Nuodex 72 for test. Flamboyán wood impregnated with a 0.05-percent solution of Nuodex 72 was eaten in 10 days; the 0.1-percent in 34 days; but for the subsequent 5 years the sample impregnated with the 0.2-percent solution of this coconutamine salt of tetrachlorphenol resisted termite attack. At the same time a sample of Nuodex 136 was submitted; flamboyán wood impregnated therewith at a 0.1-percent level was eaten in 9 days, but the 0.2-percent sample remained immune to termite attack for nearly 6 years. The manufacture and sale of this chemical, zinc petroleum sulfonate, has been taken over by the Cellu-san Division, Dalworth Inc., Simbsbury, Conn. Both of these chemicals are not only available commercially, but had been formulated for commercial impregnation of wood to protect against fungi and bacteria before their value against termites was recognized.

Nearly 6 years ago, when the chemicals synthesized by Julius Hyman still bore only his code numbers, and Aldrin was 118 and Dieldrin 497, small samples were made available for test through the courtesy of Mortimer D. Leonard, a former employee of the Station. These were intended for use against white grubs, in the control of which Aldrin proved very effective, but a small part of the experimental samples was used for the impregnation of flamboyán wood. From the very start these chemicals proved very repellent, the samples impregnated with 0.02-percent Aldrin not being eaten until 289 days had elapsed, and with 0.02-percent of Dieldrin, 201 days. Wood samples impregnated with the 0.5-percent solution were not attacked for nearly 3 years, those at 1 percent remained immune. The record of these particular chemicals is not so remarkable, however, as that of Hyman 49-RL·268, now called Isodrin, and Hyman 49-RL-269, now called Endrin, which, like the gamma isomer of BHC, as compared with the other isomers, differ in only one characteristic from Aldrin and Dieldrin, yet show a remarkable difference in the permanence with which they repel termite attack. The flamboyán sample impregnated with a 0.02-percent solution of

Endrin was eaten in 752 days, and at 0.05-percent remained entirely resistant to attack for nearly 5 years; the sample impregnated with 0.02 per cent of Isodrin was eaten in 758 days; at 0.05 percent it was immune to termite injury 5 years later.

Aldrin is 1,2,3,4,10,10-hexachlor-1,4,4*a*,5,8,8*a*-hexahydro-1,4-endo, exo-5,8-dimethanonaphthalene; Isodrin is 1,2,3,4,10,10-hexachlor-1,4,4*a*,5,8,8*a*-hexahydro-1,4-endo, endo-5,8-dimethyanonaphthalene; Dieldrin is 1,2,3,4,10,10-hexachloro-6-7-eposy-1,4,4*a*,5,6,7,8,8*a*-octahydro-1,4-endo, exo-5,8-dimethanonaphthalene; Endrin is 1,2,3,4,10,10-hexachloro-6,7-eposy-1,4,4*a*,5,6,7,8,8*a*-octahydro-1,4-endo, endo-5,8-dimethanonaphthalene.

DISCUSSION

The large number of compounds tested which had less than the value of pentachlorphenol in their permanence of resistance to termite attack have been cited only for the record. As far as dry-wood termites are concerned, they are valueless. The exception of citing DDT is made only because of publicity given to that chemical for other insecticidal purposes. Indeed, it is only for the record that the chemicals merely "as good as" pentachlorphenol are mentioned at all, for, unless they are competitive on a price basis, they can hardly be recommended in preference that well-known and widely tested chemical. In the same way, those chemicals which are merely "as good as" sodium pentachlorphenate and copper pentachlorphenate are noted primarily for the record. But those chemicals, some of which are only experimentally available, which for the longer periods of test are clearly more effective in dry-wood termite control, are certainly worthy of earnest consideration by any insecticide manufacturer. Even those which have been tested only a short time merit consideration, especially if they can be synthesized at low cost when produced on a commercial scale. Indeed, these most effective chemicals may actually prove to be the cheapest on a price basis, costing less than the solvent in which they are dissolved. Of the compounds of which only the date on which they were eaten is reported, this is within such a short time before the date of correction of proof that it may be considered the *known* length of time that the next greater concentration has resisted attack. Whether the sample with the next greater concentration will be eaten within a few days, weeks, months, years, or never, cannot be predicted, and should not be assumed on the basis of supposed similarity to some other chemical.

RESISTANT AT 2-PERCENT IMPREGNATION

DDT: 1-percent impregnated on almácigo wood attacked in 38 days; 2-percent uneaten in 10.5 years.

RESISTANT AT 1-PERCENT IMPREGNATION

Pentachlorphenol (Monsanto): 0.5-percent-solution impregnated on almácigo wood attacked in 273 days; 1-percent slightly eaten in 337 days, not subsequently eaten in 11 years.

Pyridyl mercuric stearate (Mallinckrodt): 0.5-percent eaten in 1,241 days; 1-percent uneaten after 9 years.

Tectoquinone (β-methylanthraquinone): 0.5-percent eaten in 536 days; 1-percent uneaten after 9 years.

Zinc lake (acetic acid) *of β-chloranthraquinone:* 0.5-percent eaten in 96 days; 1-percent uneaten after 6 years.

Zinc lake (acetic acid) *of quinizarin:* 0.5-percent eaten in 96 days; 1-percent uneaten after 6 years.

Diphenyl mercury: 0.5-percent eaten in 176 days; 1-percent uneaten after 8.5 years.

Piperonyl cyclohexenone (Dodge & Olcott): 0.5-percent eaten in 219 days; 1-percent uneaten after 7 years.

4,6-Dinitro-o-sec-butylphenyl (Dow); 0.5-percent eaten in 244 days; 1-percent uneaten after 7 years.

Xanthone (Laurel Hill): 0.5-percent eaten in 1,053 days; 1-percent uneaten after 6.5 years.

Genecide acids (Laurel Hill): 0.5-percent eaten in 163 days; 1-percent uneaten after 6.5 years.

Dimethyl tetrachlorphthalate (Penn Salt): 0.5-percent eaten in 5 years; 1-percent uneaten after 6 years.

Dinitro-o-phenyl phenol: 0.5-percent eaten in 69 days; 1-percent uneaten after 6 years.

4-Iodox xanthone (Laurel Hill): 0.5-percent eaten in 124 days; 1-percent uneaten after 6 years.

Cupric salt of 3-penylsalicylic acid (Dow: K-4738): 0.5-percent eaten in 79 days; 1-percent uneaten after 6 years.

2,4-Dichlorphenyl-2-phenyl benzoate (Laurel Hill): 0.5-percent eaten in 126 days; 1-percent uneaten after 6 years.

Dihydropinosylvin monomethyl ether (Erdtman): 0.5-percent eaten in 1,279 days; 1-percent uneaten after over 5 years.

β-Chlorethyl-β-(p-tert-butyl phenoxy) β-methyl ethyl sulfite, "Aramite" (Naugatuck): 0.5-percent eaten in 1,632 days; 1-percent uneaten after 5 years.

1,1-Di(4-hydroxy-2,5-dichlorphenyl) 2,2,2-trichlorethane (Laurel Hill): 0.5-percent eaten in 615 days; 1-percent uneaten after nearly 5 years.

Aldrin (Hyman 118) Shell: 0.5-percent eaten in 1,230 days; 1-percent uneaten after nearly 6 years.

Dieldrin (Hyman 497) Shell: 0.5-percent eaten in 1,230 days; 1-percent uneaten after nearly 6 years.

RESISTANT AT 0.5-PERCENT IMPREGNATION

Sodium pentachlorphenate (Monsanto): 0.2-percent eaten in 746 days; 0.5-percent uneaten after 8 years.

2-Methyl xanthone (Laurel Hill): 0.2-percent eaten in 200 days; 0.5-percent uneaten after 6 years.

4-Methyl xanthone (Laurel Hill): 0.2-percent eaten in 182 days; 0.5-percent uneaten after 6 years.

Zinc lake (acetic acid) *of Anthrarufin and Chrysazin:* 0.2-percent eaten in 96 days; 0.5-percent uneaten after 8 years.

Ammonium dinitro-ortho-sec-butylphenol: 0.2-percent eaten in 59 days; 0.5-percent uneaten after 6 years.

1,1-Di(2-hydroxy-3,5-dichlorphenyl) 2,2,2-trichlorethane (Laurel Hill): 0.2-percent eaten in 1,895 days; 0.5-percent uneaten after over 5 years.

Hexachlorcyclopentadiene dimer (Laurel Hill): 0.2-percent eaten in 166 days; 0.5-percent uneaten after 5 years.

Oxygenated hexachlorcyclopentadiene dimer (Laurel Hill): 0.2-percent eaten in 675 days; 0.5-percent uneaten after 5 years.

2-Nitro-1,1-bis(p-chlorphenyl)propane (Commercial Solvents): 0.2-percent eaten in 240 days; 0.5-percent uneaten in over 5 years.

Chlorophorin (King of Nottingham U.): 0.2-percent eaten in 234 days; 0.5-percent uneaten after 4 years.

Pinosylvin dimethyl ether (Erdtman): 0.2-percent eaten in 577 days; 0.5-percent uneaten after 3 years.

Ortho-di-chlorbenzene fumaril chloride (Laurel Hill): 0.2-percent eaten in 47 days; 0.5-percent uneaten after nearly 3 years.

2,4,2',4'-Tetranitrostilbene (Erdtman): 0.2-percent eaten in 2 years, 4 months.

Dibenzoyl ethylene (Laurel Hill): 0.2-percent eaten in 341 days; 0.5-percent uneaten after 2.5 years.

Di-2-ethylhexyl-2,3,4,5-tetrachorphthalate (Penn Salt): 0.2-percent eaten in 659 days; 0.5-percent uneaten after 2 years.

Glycol (alkyd) resin 2,3,4,5-tetrachlorphthalate (Penn. Salt): 0.2-percent eaten in 69 days; 0.5-percent uneaten after 2 years.

Phenol heptachlor propane (Laurel Hill): 0.2-percent eaten in 675 days; 0.5-percent uneaten after 2 years.

1,1-Bis(p-ethylphenyl) 2,2-dichlorethane (Rohm & Haas Q-137): 0.2-percent eaten in 652 days; 0.5-percent uneaten after over 2 years.

Coconutamine salt of tetrachlorphenol (Nuodex 72) Nuodex Products: 0.1-percent eaten in 34 days; 0.2-percent eaten in 5 years, 4 months.

Zinc petroleum sulfonate (Nuodex 136) "Cellu-san" Darworth Inc.: 0.1-percent eaten in 9 days; 0.2-percent eaten in 5 years, 10 months.

RESISTANT AT 0.2-PERCENT IMPREGNATION

Copper pentachlorphenate (Monsanto): 0.1-percent eaten in 11 days; 0.2-percent uneaten after 9 years.

Pyridyl mercuric chloride (Mallinckrodt): 0.1-percent eaten in 27 days; 0.2-percent uneaten after 9 years.

2-Nitro-1,1-bis (p-chlorphenyl) butane (Commercial Solvents): 0.1-percent eaten in 335 days; 0.2-percent uneaten after 4.5 years.

Vulpinic (lichen) acid (Erdtman): 0.1-percent eaten in 159 days; 0.2-percent uneaten after 3 years.

Azobenzene (Erdtman): 0.2-percent eaten in 3 years, 2 months.

2,4-Dichlorstilbene (Erdtman): 0.1-percent eaten in 277 days; 0.2-percent uneaten after 2.5 years.

0.0-Diethyl-0-(2-isopropyl-4-methyl-pyrimidyl-6) thionphosphate "Diazinon" (Geigy): 0.1-percent eaten in in 2 years.

RESISTANT AT 0.1-PERCENT IMPREGNATION

1,1-Di(4-hydroxy-2-methyl-5-isopropyl) 2,2,2-trichlorethane (Laurel Hill): 0.05-percent eaten in 38 days; 0.1-percent uneaten in less than 2 years.

RESISTANT AT 0.05-PERCENT IMPREGNATION

Methoxychlor 1949 (du Pont); *2,2-bis(p-methoxyphenyl)1,1,1-trichlorethane:* 0.02-percent eaten in 1 year; 0.05-percent uneaten after 5 years.

Isodrin: 1,2,3,4,10,10-hexachlor-1,4,4a,5,8,8a-hexahydro-1,4-endo, endo-5,8-dimethanonaphthalene (Hyman 49–RL-268): Shell: 0.02-percent eaten in 758 days; 0.05-percent uneaten after nearly 5 years.

Endrin: 1,2,3,4,10,10-hexachlor-6,7-eposy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo, endo-5,8-dimethanonaphthalene (Hyman 49-RL-269) Shell: 0.02-percent eaten in 752 days; 0.05-percent uneaten in nearly 5 years.

Prentox "Strobane" (B. F. Goodrich): 0.01-percent eaten in 564 days; 0.02-percent eaten in less than 2 years.

Ryanodine: (originally Merck, now S. B. Penick): 0.02-percent eaten in 204 days; 0.05-percent slightly eaten after 5 years.

Taxifolin (Erdtman): 0.02-percent eaten in 154 days; 0.05-percent uneaten after 3.5 years.

2,3,4,5-Tetrachlorbenzoic acid (Penn Salt): 0.02-percent eaten in 54 days; 0.05-percent uneaten in 2 years, 7 months.

2,4-Dihydroxyphenanthrene (Erdtman): 0.05-percent eaten in 594 days.

RESISTANT AT 0.01-PERCENT IMPREGNATION

Pinosylvin monomethyl ether (Erdtman): 0.005-percent untested; 0.01-percent uneaten after 4.5 years.

1,1-Di(4-hydroxy-2-methyl-5-chlorphenyl)2,2,2-trichlorethane (Laurel Hill): 0.005-percent eaten in 19 days; 0.01-percent uneaten in 2 years, 3 months.

2,4-Dihydroxybenzalaniline (Erdtman): 0.002-percent eaten in 257 days; 0.005-percent eaten in 3 years, 2 months.

RESISTANT AT 0.005-PERCENT IMPREGNATION

2,4-Dinitro-2',4'-dichlorstilbene (Erdtman): 0.002-percent untested; 0.005-percent uneaten after 4.5 years.

4,4'-Dinitrostilbene (Erdtman): 0.005-percent uneaten after 4.5 years.

SUMMARY

The high resistance to decay and insect attack of some woods which makes them of such great value for building and the construction of furniture for use in the Tropics, is due to the presence in the heartwood of each species of a comparatively minute amount of some unique chemical. Such chemicals appear to have little or no survival value for the tree itself, and indeed often result in its early elimination by selective cutting in virgin tropical forests. The less desirable woods, however, may be given at least superficial protection by painting, spraying, submergence, or pressure-impregnation with such extracted constituents of resistant woods, or with other chemicals synthesized in the laboratory.

If not leached out by water, the protection afforded by such water-soluble chemicals as copper sulfate or zinc chloride and most repellent inorganic chemicals is permanent, but that resulting from surface impregnation of organic chemicals, because of their inherent volatility or instability, is often only temporary. Thus permanent effectiveness is of even greater importance than the concentration of the chemical used for wood impregnation, and for the present summarization is barely mentioned, unless for a greater period than 5 years, with full realization that even 5 years is far from being the equivalent of forever.

In preventing attack on such termite-susceptible woods as almácigo and flamboyán by the West Indian dry-wood termite, *Cryptotermes brevis* Walker, submergence for 10 minutes in a 2-percent solution of DDT has been effective twice for over 5 years.

A 1-percent solution of commercial pentachlorphenol impregnated into the wood has protected almácigo for 11 years; and for somewhat shorter periods so did the teak extractive tectoquinone; the zinc lakes (acetic

acid) of quinizarin and of β-chloranthaquinone; piperonyl cyclohexenone (Dodge & Olcott); 4,6-dinitro-o-sec-butylphenyl (Dow); dinitro-o-phenyl phenol (Dow); xanthone, 4-iodox xanthone, and genicide acids; 2,4-dichlorphenyl-2-phenyl benzoate (Laurel Hill); dimethyl tetrachlorphthalate (Penn Salt); Dow's K-4738; a cupric acid salt of 3-phenyl-salicylic acid; dihydropinosylvin monomethyl ether (Erdtman); Naugatuck's miticide "Aramite," and Shell's Aldrin and Dieldrin.

An impregnation of one-half of 1 percent of sodium pentachlorphenate (Monsanto) has protected almácigo wood against termite attack for 8 years, as did also that of the zinc lake (acetic acid) of anthrarufin and chrysazin. Similar protection for 5 to 6 years was afforded flamboyán wood by ammonium dinitro-ortho-sec-butylphenol, or "Sintox", of Standard Agricultural Chemicals. The following experimental chemicals synthesized at the Laurel Hill Laboratories: 2-methyl and 4-methyl xanthone, hexachlorcyclopentadiene dimer and its oxigenated dimer, and 1,1-di(2-hydroxy-3,5-dichlorphenyl)2,2,2-trichlorethane, did the same. Two Nuodex Products: coconutamine salt of tetrachlorphenol (Nuodex 72) and zinc petroleum sulfonate (Nuodex 136), now renamed "Cellu-san", have done likewise for over 5 years on flamboyán.

An impregnation of one-fifth of 1 percent of copper pentachlorphenate (Monsanto) has protected almácigo wood against termite attack for 9 years.

An impregnation of one-twentieth of 1 percent of 1949 Methoxychlor (duPont), 2,2-bis(*p*-methoxyphenyl)1,1,1-trichlorethane, has protected flamboyán against termite attack for 5 years, as did also Isodrin and Endrin (Shell) synthesized by Julius Hyman, and the extractive of *Ryania speciosa*, Ryanodine (Ryania of Merck, now of S. B. Penick).

Of the extractive pinosylvin monomethyl ether from *Pinus sylvestris*, the impregnation on flamboyán of one-hundredth of 1 percent has not been attacked by termites in 4.5 years. Of the other experimental chemicals synthesized in the Laboratory of Organic Chemistry, the Royal Institute of Technology, Stockholm, Sweden, the two-hundredth of 1-percent impregnation of 2,4-dihydroxybenzalaniline resisted termite attack for 3 years, 2 months before being eaten, while at this extreme dilution, 4,4′-dinitrostilbene and 2,4-dinitro-2′,4′-dichlorstilbene are still immune from termite attack 4,5 years after impregnation.

RESUMEN

La alta resistencia a los ataques de los insectos y a podrirse de cada madera, propiedades necesarias para darle valor como material de construcción y para la manufactura de muebles en los trópicos, se deben a la presencia de comparativamente pequeñas cantidades de alguna substancia química específica para cada especie de árbol. Estos agentes químicos aparentemente tienen muy poco valor, o quizás ninguno, en lo que se refiere a

la supervivencia del árbol en sí, y casi siempre los arboles desaparecen pronto durante el corte selectivo en los bosques vírgenes tropicales. A las maderas menos deseables, sin embargo, podrían dárseles, por lo menos protección superficial, pintándolas, asperjándolas, sumergiéndolas o impregnándolas a presión con los constitutivos extraídos de las maderas resistentes, o con otros compuestos químicos sintetizados en el laboratorio.

A menos que no se lave con el agua, la protección adquirida por medio de estos compuestos inorgánicos específicos, es permanente. No resulta así la protección que adquieren las maderas por medio de la impregnación superficial con agentes químicos orgánicos, ya que la volatilidad inherente o su inestabilidad es frecuentemente temporera. Por este motivo, la *permanencia* de la eficacia del agente químico es aún de mayor importancia que el grado de *concentración* del compuesto que se use para impregnar la madera. Claro está, que la eficiencia del tratamiento debe durar por mucho más tiempo que 5 años para poder considerarla recomendable. Se apunta aquí que cuando se sumergieron el almácigo y el flamboyán, maderas muy susceptibles a los ataques de la polilla de la madera seca, *Cryptotermes brevis* Walker, por 10 minutos en una solución al 2 por ciento de DDT, los efectos preservativos duraron por 10 años.

El pentaclorofenol comercial, a una concentración del 1 por ciento ha protegido la madera de almácigo por 11 años después de impregnada; por otros períodos más cortos, la madera de almácigo ha sido protegida por los siguientes agentes químicos: extractivo tectoquinona de teca; piperonil cyclohexenona (Dodge & Olcott); 4,6-dinitro-o-sec-butilfenol (Dow); pigmentos de zinc (acido acético) de quinizarína y de beta-clorantaquinona; dinitro-o-fenilfenol (Dow); xantona, 4-iodo-xantona, y los ácidos gentícicos 2,5-dichloryfeniol-2-fenolbenzoato (Laurel Hill); dimetyl tetracloroptalato (Penn Salt); K-4738 de Dow; sal cúprica del ácido 3-fenolsalicílico; éter dihidropinosilvino monometílico (Erdtman); miticido "Aramite" de Naugatuck; y Aldrín y Dieldrín de Shell.

Una impregnación de medio por ciento de pentaclorofenato de sodio (Monsanto) ha protegido la madera de almácigo contra la polilla por 8 años, al igual que con una de pigmento de zinc (ácido acético) de antrarufín y crysazín. La madera de flamboyán fué protegida, de 5 a 6 años, cuando se impregnó con el dinitro-orto-sec-butilfenol de amoníaco, conocido por "Sintox W" de la "Standard Agricultural Chemicals" y con los agentes químicos sintetizados por los laboratorios "Laurel Hill", a saber: 2-metilo y 4-metilo xantona; dímero de hexaclorociclo pentadieno y su dímero oxigenado; y 1,1-di (2-hidroxi-3-5-diclorifenil) 2,2-tricloretano.

Pudo protegerse la madera de almácigo de los ataques de la polilla por 9 años cuando se impregnó con una solución que tenía una quinta parte de un por ciento de pentaclorofenato de cobre (Monsanto).

La madera de flamboyán se logró proteger por 5 años impregnándola con dos productos "Nuodex"; la sal coconutamina de tetraclorofenol (Nuodex 72) y el petróleo sulfonato de zinc (Nuodex 136), ahora llamado "Cellusan".

En el 1949, la madera de flamboyán impregnada con $\frac{1}{20}$ de un por ciento de metoxyclor (Dupont), 2,2-bis (p-metoxifenil) 1,1,1,-tricloroetano, conservó su resistencia por 5 años. Igual pasó cuando se impregnó con Isodrín y Endrín (Shell), ambos productos sintetizados por el Dr. Julius Hyman, y con el extractivo de *Ryania speciosa*; ryanodina ("Ryania" Merck, ahora de S. B. Penick).

El extractivo de *Pinus sylvestris*, éter monometilo de pinosilvino de los compuestos químicos 2′,4′,2′,4′,-tetranitrostilbeno, sintetizados por los laboratorios del Dr. Erdtman en Suecia, cuando se usaron para impregnar la madera de flamboyán, a razón de $\frac{1}{100}$ de un por ciento, esta resistió los ataques de la polilla por 4½ años. La misma madera impregnada con los compuestos 2,4-dinitro-2′,4′-diclorostilbeno y 4,4′-dinitrostilbeno, a razón de $\frac{1}{200}$ de un por ciento ha resistido hasta ahora por 4½ años.

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The Mechanism of Apomixis in *Pennisetum Ciliare*

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INTRODUCTION

Pennisetum ciliare (L.) Link is an extremely polymorphic species having an extensive native range which extends over much of Africa, Madagascar, Sicily, and eastward to Palestine, India, and Java. The species has been introduced into Australia and the Americas. In Puerto Rico it is now a major component of the flora in savanna areas of the semiarid southern coastal region (2)[4]. *P. ciliare* has recently received appreciable attention in some areas of the southern United States because of the occurrence of desirable forage ecotypes adapted to arid and semiarid habitats. Interest in the species in Puerto Rico has resulted primarily from the introduction of strains agronomically superior to those already established on the Island.

Meiotic irregularities encountered in a study of microsporogenesis in a number of introduced strains of *P. ciliare*, and the marked uniformity of progenies grown from bulk-collected seed which originated from a number of different localities, initially suggested the possibility of apomictic reproduction within this highly variable species. The preliminary study of sectioned material of developing ovules indicated the occurrence of appreciable irregularity, and the present investigation was undertaken to study the mechanism of reproduction in the species, with particular reference to megasporogenesis and megagametogenesis.

MATERIAL AND METHODS

Seeds of 13 strains of *P. ciliare*, representing various parts of the native range of the species, were obtained through the cooperation of Edwin James of the Southern Regional Plant Introduction Station, Experiment, Ga. The initial observations were made on field-grown plants of these strains and plants of a "native" strain which originated from material collected near Salinas, P. R., by Ovidio García Molinari. The principal source of material for the present investigation was a strain of *P. ciliare* collected near Tamale, Gold Coast (P.I. 185641)[5]. Supplementary data were obtained

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[4] Numbers in parentheses refer to Literature Cited, p. 164.

[5] P.I. numbers refer to accession numbers of the Section of Plant Exploration and Introduction, USDA.

from material of the Puerto Rican strain and from strains originating in Southern Rhodesia (P.I. 156540 and 203521), Ethiopia (P.I. 197443), Morocco (P.I. 202513), East Punjab, India (P.I. 164414), and Western Australia (P.I. 193445).

Although *P. ciliare* is an extremely polymorphic species, most of the strains studied, as well as a number of additional strains growing in the same nursery, exhibited marked intrastrain uniformity. Two exceptions, which might be expected in view of the fact that the progenies were grown from bulk-collected seeds, were P.I. 164414 and 202513. The culture of the latter strain included plants of two distinctly different morphological types, both of which were identified as *P. ciliare*. The culture of P.I. 164414 contained plants of at least three different morphological types, one of which was identified by Ernest R. Sohns, of the U. S. National Herbarium, as *Cenchrus setigerus* Vahl. Because of the similarity of this material (considered part of the same agamic complex by Fisher, *et al.* (1) pertinent data from this species have been treated along with *P. ciliare*. Herbarium specimens of the various strains of *P. ciliare* involved in the investigation, and of the *C. setigerus* material, have been deposited in the U. S. National Herbarium.

Immature inflorescences for the study of microsporogenesis were fixed in a 3:1 mixture of 95-percent alcohol and glacial acetic acid for 24 hours and then washed and stored in 70-percent alcohol. Individual florets were not dissected from the inflorescences, but the material was aspirated immediately after being placed in the fixative to facilitate penetration of the solution. Analyses of meiotic behavior were made from temporary iron-aceto-carmine smears.

Material for the study of megasporogenesis and megagametogenesis was killed in formalin-acetic-alcohol (70-percent alcohol) after the florets had been dissected from the inflorescences and the distal one-third of the lemma and palea had been out off to facilitate penetration of the fixative. For stages after anthesis, the glumes and lemma were removed prior to embedding, because the nerves of the second glume and the lemma become sufficiently indurate at this stage to cause difficulty in sectioning. The florets were embedded in paraffin by the normal-butyl alcohol method. Sectioning was done at 15 micra, and the material was stained in from hematoxylin.

Pollination studies were made with inflorescences that had been bagged about 56 hours before anthesis. Bagging was necessitated by the fact that the stigmas of *P. ciliare* florets are exserted and apparently receptive as early as 36 to 48 hours before anthesis. The bags were removed at the time of anthesis, and all florets whose anthers had not dehisced within 10 minutes after the first pollen was shed were removed from the inflorescence. The stigmas were removed completely from the remaining florets at various

intervals after anthesis. Examination of the treated florets to determine the percentages of seeds set was made prior to maturity and shattering of the inflorescence.

OBSERVATIONS

MICROSPOROGENESIS

Hernández (3) has reported chromosome numbers of $2n = 36$ for five strains of *P. ciliare*, including a strain from Puerto Rico, and $2n = 54$ for a sixth strain. Fisher, *et al.* (1) investigated strains of the species with chromosome numbers of $2n = 32$, 36, 40, and 54, and found $2n = 36$ in all material of *C. setigerus* that they studied. Incidental to a study of microsporogenesis in several strains of *P. ciliare*, we have verified the number $2n = 36$ in two of the collections reported earlier by Hernández, the Puerto Rican strain and P.I. 193445, and have found the same number in three other unreported collections, P.I. 185641, 197443, and 203521. In addition, a single plant of P.I. 202513 was studied and found to have $2n = 43$, and two plants of P.I. 156540 exhibited $2n = 48$. The only material of *C. setigerus* studied in the present investigation was from the collection P.I. 164414, and had the chromosome number $2n = 36$.

Meiotic behavior was studied in four plants of P.I. 185641, the strain from which the major part of the material used in the investigation of megasporogenesis and megagametogenesis was obtained. Although Fisher, *et al.* (1) reported no irregularity at meiosis in the 36-chromosome types, those investigated in the present study showed considerable irregularity. Most of the microsporocytes examined at diakinesis and first metaphase contained 2-4 quadrivalents. The maximum chromosome association observed in an aggregate sample of several hundred cells was $5_{IV} + 8_{II}$. The mean numbers of various chromosome configurations, based on the analysis of 106 diakinesis cells from one of the plants, were: 2.7_{IV}, 0.1_{III}, 12.0_{II}, and 0.9_{I}. From 1 to 5 univalents were found in 39 percent of the cells analyzed. Lagging chromosomes were present in 36 percent of the first anaphase figures examined, with usually only one or two univalent chromosomes exhibiting lagging in a given cell. Of 59 late anaphase figures in which the complete chromosome complement could be counted, and which showed no evidence of lagging chromosomes, 34.5 percent were found to have unbalanced distributions of the 36 chromosomes, i.e., 17 to 19 and 16 to 20 disjunctions. The second meiotic division exhibited greater regularity than did the first, and only 14 percent of the sporads (or tetrads of microspores) examined contained micronuclei. Chromosome pairing and the other aspects of meiosis were very similar in the four plants studied, and pollen stainability in iodine-potassium iodide or acetocarmine was 52 to 60 percent in each of the plants.

Chromosome association at diakinesis and first metaphase in plants of the five additional 36-chromosome strains was found to be essentially comparable to that in P.I. 185641, although some variation was observed from strain to strain. The frequencies of first anaphase figures with lagging chromosomes were within the range of 30 to 40 percent for P.I. 193445, 197443, and 203521. On the other hand, the Puerto Rican strain and P.I. 164414, both of which had lower univalent frequencies than the other 36-chromosome strains, had lagging chromosomes in only 10 to 14 percent of the first anaphase cells examined. Satisfactory material for the study of nondisjunction was available only for the Puerto Rican strain. In this strain the frequency of late first anaphase figures showing a deviation from the normal 18 to 18 distribution was approximately 13 percent, appreciably lower than was found in P.I. 185641. As in the case of the latter strain, the second meiotic division in the other 36-chromosome strains was more regular than the first. The percentages of sporads with micronuclei were within the range of 5 to 16 percent, and pollen stainabilities ranged from 50 to 70 percent in plants of all of these strains.

Meiosis in the 43- and 48-chromosome plants was found to be relatively much less regular. Chromosome association at diakinesis and first metaphase in the 43-chromosome plant differed from that in the 36-chromosome plants primarily in a higher univalent frequency. Each of the 53 diakinesis cells analyzed contained one to nine univalents, and the mean frequency was 4.7 univalents per cell. Lagging chromosomes at first anaphase were found in 97 percent of the cells studied, and most of the cells contained 3 to 7 laggards. Second anaphase showed only slightly less irregularity than did the first, and 96 percent of the sporads contained one or more micronuclei. The mature pollen grains, of which 18 percent were stainable with IKI or acetocarmine, exhibited an appreciable variation in size.

The maximum chromosome association observed at diakinesis in the 48-chromosome plants was $14_{IV} + 16_{II}$, and several cells were found with $5_{IV} + 12_{II} + 4_{I}$. On the basis of a limited sample of 21 diakinesis cells that could be completely analyzed, mean chromosome association in one of the 48-chromosome plants was 3.2_{IV}, 0.2_{III}, 13.3_{II}, and 7.4_{I}. Approximately 98 percent of the first anaphase figures studied in each of these plants contained lagging chromosomes, with most cells having 5 to 14. The formation of micronuclei, commonly 6 to 12 per sporad, was found to occur in 95 to 98 percent of the sporads examined. Pollen-stainabilities of the two plants from which meiotic material was studied, and of three other plants of the same collection, were 5 to 9 percent.

Although meiosis in the aneuploid plants is relatively irregular, no evidence of aberrations in the spindles or in cytokinesis was found. The cell wall separating the two members of a first-division dyad is formed prior to

the onset of the second division. The two second-division figures are essentially parallel to each other, resulting in the formation of the usual sporad or tetrad of microspores encountered in most monocotyledons.

MEGASPOROGENESIS

Differentiation of the archesporial cell from a hypodermal cell of the nucellus occurs at about the time the primordium of the inner integument is first apparent. At this stage the ovule is turned at an angle of about 90° to the longitudinal axis of the floret. With subsequent growth the ovule rotates until it approaches an anatropous condition. After a period of elongation (fig. 1, A), during which the inner integument envelopes most of the nucellus and the outer integument becomes well-defined, the archesporial cell occupies the full depth of the nucellus. During this period also there is a proliferation of the cells in the micropylar region of the nucellar epidermis by one or more periclinal divisions. The outer cells in this region subsequently undergo a marked increase in size (fig. 1, B, *et. seq.*). This enlargement also was noted by Fisher, *et al.* (1).

The archesporial cell functions directly as the primary megasporocyte, and meiosis is initiated at about the same time that it is in microsporocytes of the same floret. Although observations were necessarily limited to a relatively small number of division figures, the first meiotic division appears to be comparable to that in microsporogenesis and results in the formation of a well-defined dyad (fig. 1, B). The chalazal member of the dyad subsequently undergoes a normal second meiotic division (fig. 1, C), which frequently fails to occur, or is not completed, in the micropylar cell (fig 1, D). Meiosis may thus result in the formation of a linear tetrad, a linear triad, or rarely in a T-shaped tetrad of megaspores, depending upon the occurrence, completeness, and plane of the second meiotic division in the micropylar member of the dyad. The products of the meiotic divisions, with the usual exception of the chalazal megaspore, undergo a rapid degeneration soon after they are formed. The degenerating cells can be detected readily because of their shrunken appearance and dense stainability with hematoxylin (fig. 1, E, F).

APOSPORY

One or more enlarging nucellar cells, usually adjacent to the sporogenous material, frequently can be distinguished at the time of megasporogenesis, or shortly thereafter (fig. 1, F). In an occasional ovule the chalazal megaspore undergoes an early and presumably autonomous degeneration before the nucellar cells have enlarged very much. More typically, this megaspore persists as the nucellar cells enlarge. The megaspore characteristically lags in development, however, and often exhibits evidence of degeneration by

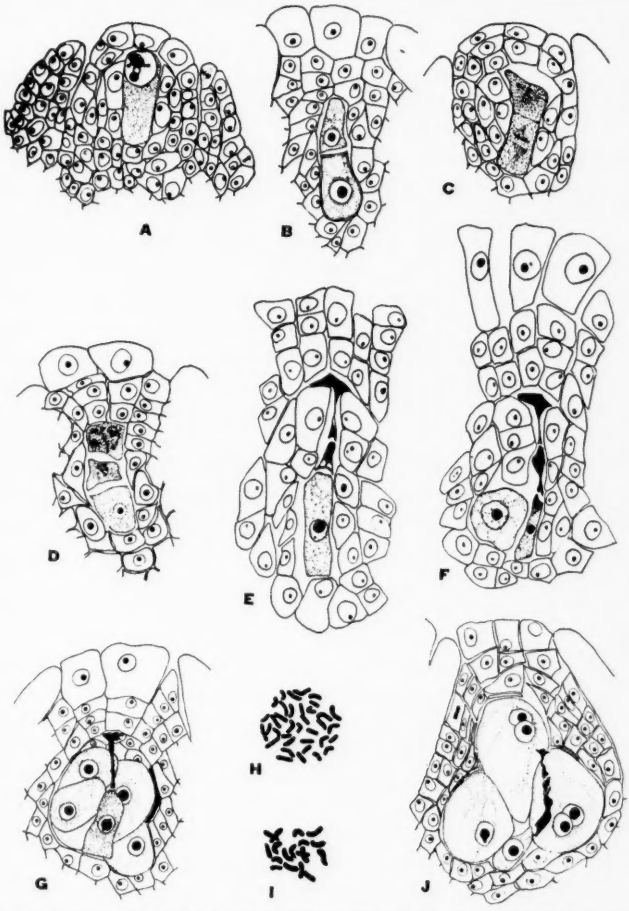

FIG. 1.—Camera lucida drawings of megasporogenesis and apospory in *Pennisetum ciliare*: A, Mature archesporial cell. B, Dyad resulting from first meiotic division. C, Second meiotic division. Note orientation and delay of division in micropylar cell. D, Second division complete in basal dyad, forming chalazal and adjacent megaspores. Division of micropylar dyad is incomplete. E, Chalazal megaspore enlarged, other megaspores degenerating. F, Degeneration of megaspores (including basal one) accompanied by enlargement of adjacent somatic cell of nucellus. G, Four enlarged nucellar cells and apparently normal basal megaspore. H, Unreduced chromosome number, $2n = 36$, observed in mitotic division of enlarging nucellar cell. I, Reduced number of chromosomes, $n = 18$, from mitotic division of functional megaspore. J, Three young aposporic embryo sacs and remnants of megaspores. All except H and I ×450; H and I ×1800.

the time the nucellar cells have undergone an appreciable increase in size (fig. 1, F, G). The chalazal megaspore can occasionally be seen, sometimes with the recognizable remains of the other sporogenous material, in ovules in which several nucellar cells and/or aposporic embryo sacs have attained appreciable development (fig. 1, J).

There are striking differences in appearance between persistent megaspores and enlarging nucellar cells. The former may exhibit a limited early increase in size, and infrequently an appreciable increase in size, but tend to remain elongate and the cytoplasm maintains a uniform appearance (fig. 1, G, J). The nucellar cells, on the other hand, rapidly increase in size and develop a rounded shape and highly vacuolate cytoplasm at an early stage. This is typical of aposporous behavior in many apomictic species. There is usually pronounced crowding and distortion in the region of the sporogenous material as the nucellar cells enlarge, which makes accurate interpretation difficult or impossible in many ovules. Observation of the stages immediately after formation of the megaspores in a large number of ovules of the various strains studied, indicates that development of a chalazal megaspore into a 2-nucleate embryo sac is a relatively rare occurrence in most of this material. Evidence which will be presented below, however, points to some variation among different strains in frequency of functioning of the megaspore.

An additional indication that the embryo sacs are predominantly derived from aposporic initials is found in the chromosome numbers of dividing embryo sac nuclei. Division figures were found in seven developing embryo sacs of P.I. 185641 and in five additional embryo sacs of the other strains studied. The diploid chromosome number, $2n = 36$, (fig. 1, H) could be counted in the mitotic figures in five of these embryo sacs, and in six of the remaining embryo sacs it was possible to obtain counts which approximated the diploid chromosome number. The only 4-nucleate embryo sac in process of division was found in P.I. 185641 and contained the reduced number of chromosomes, $2n = 18$, (fig. 1, I). The significance of this particular case, which establishes the fact that reduced embryo sacs are occasionally produced, will be discussed later.

MEGAGAMETOGENESIS

Development of the embryo sac, or female gametophyte, apparently follows two distinctly different patterns, which are closely comparable to those found in *Panicum maximum* (6). In the type of behavior that is predominantly represented in the present material, the two nuclei resulting from division in the embryo sac initial remain at the micropylar end of the developing embryo sac (fig. 2, A)—or at the end nearest the surface of the ovule in the case of widely displaced embryo sacs. A second division of these

nuclei results in a 4-nucleate embryo sac in which the nuclei remain closely associated (fig. 2, B). Differentiation in these embryo sacs, without the occurrence of a third mitotic division, results in a mature gametophyte of the *Oenothera* type, with two synergids, an egg, and a polar nucleus (fig. 2, C).

In the second type of development, the nuclei in a young 2-nucleate embryo sac separate to opposite ends of the cell, and a large vacuole develops between them. These nuclei undergo a second division to form a 4-nucleate embryo sac (fig. 2, D, left), and subsequently a third division to produce an 8-nucleate embryo sac having four nuclei at each end (fig. 2, E). The 8-nucleate embryo sacs differentiate into mature gametophytes of the *Polygonum* type, having two synergids, an egg, two polar nuclei, and three antipodals. As is common among the grasses, the antipodals may undergo one or more additional division (fig. 2, F). Stages in development of 8-nucleate embryo sacs were relatively rare in the present material, and the process is apparently less frequent than it is in *P. maximum*.

Although all of the strains studied produce multiple embryo sacs, they differ considerably in the average number of embryo sacs per ovule. Data on the numbers of embryo sacs in five strains of *P. ciliare* and a single strain of *C. setigerus*, based on the study of ovules collected shortly before anthesis, are given in table 1. The highest mean number of embryo sacs per ovule, 4.6 in a sample of 78 florets, was found in P.I. 185641. Ovules containing 6 to 8 embryo sacs were frequently observed in this material, and no ovules with single embryo sacs were found in the sample studied. The other extreme is represented by P.I. 202513, in which a majority of the 33 ovules examined contained single embryo sacs and the mean number of embryo sacs per ovule was 1.5. The latter strain was the only one having an appreciable number of sterile ovules, i.e., ovules in which no functional embryo sacs were formed, and exhibited the highest frequency of 8-nucleate embryo sacs. P.I. 202513 also differs from the other strains examined in the timing of development of the aposporic initials. Enlarging nucellar cells frequently are not well defined until some time after meiosis has been completed in the megasporocytes of this strain; whereas considerably enlarged aposporic initials are commonly found at a comparable stage of development in the other strains.

The multiple embryo sacs are usually crowded into the central part of the ovule and may occupy most of the former region of the nucellus by the time they are mature. Frequently, however, embryo sacs are found in the chalaza; in the periphery of the ovule, where they may invade and grossly disrupt the integuments; and in the funiculus, where they sometimes invade the tissue of the ovary wall. Most of these displaced embryo sacs apparently develop later than those occupying the central region of the ovule adjacent

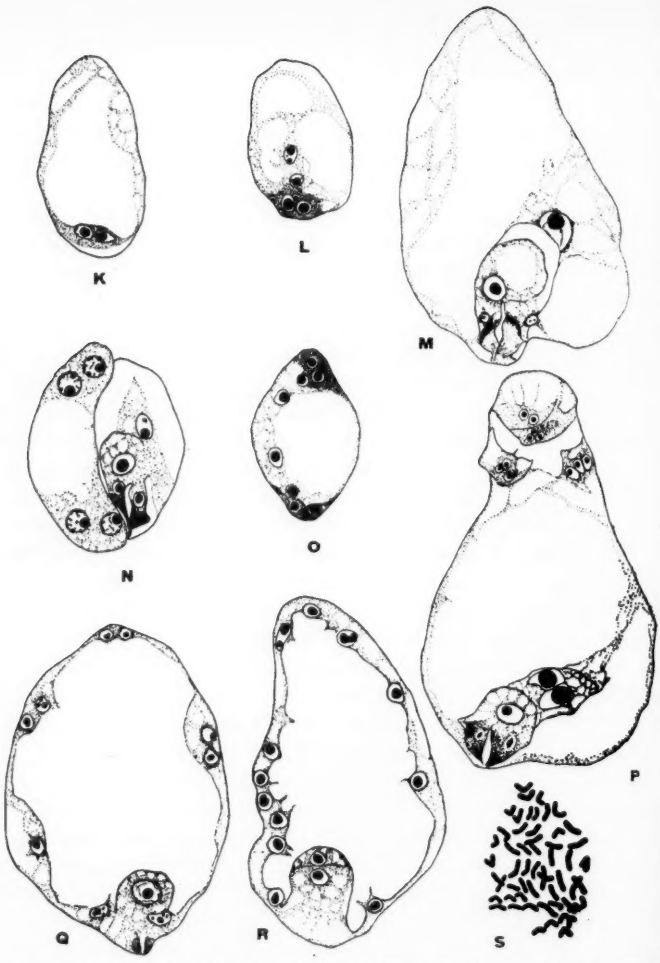

FIG. 2.—Embryo sac formation and early endosperm and embryo development in *Pennisetum ciliare*: A, B, C, Development of 4-nucleate embryo sacs: A, 2-nucleate stage, both nuclei remaining at micropylar end. B, Early 4-nucleate stage. C, Mature 4-nucleate embryo sac containing 2 synergids, 1 egg, and 1 polar nucleus. D, E, F, Development of 8-nucleate embryo sacs: D, (left) 4-nucleate embryo sac with nuclei separated to opposite ends, with each nucleus in prophase of a third mitotic division. The embryo sac to the right is aposporic and differentiated at the 4-nucleate stage. E, Young 8-nucleate embryo sac. F, Mature embryo sac of 8-nucleate type, containing 2 synergids, 1 egg, 2 polar nuclei, and 3 antipodals (which have undergone further division). G, Embryo sac subsequent to fertilization. Endosperm is 16-nucleate; egg cell has not yet divided. H, Endosperm is 64-nucleate. The pre-embryo has undergone first division. I, Fifty-four chromosomes ($3n$ number) from early division in endosperm 1800×. A, B, D, and E, 450×; C, F, G, and H, 300×.

to the original sporogenous material. The orientation of the multiple embryo sacs is an interesting phenomenon. The egg apparatus tends to be oriented toward the nearest surface of the ovule, and may be directed at a 90° angle or even a 180° angle from the micropyle, depending upon the location of the particular embryo sac. This phenomenon also was observed by Fisher, *et al.* (1). Many embryo sacs, apparently often those in which development is initiated after several large embryo sacs are already present in the ovule, are crowded out by competition and fail to reach maturity.

PSEUDOGAMY

The structure of the inflorescence and size of the florets in *P. ciliare* make the effective emasculation of large numbers of florets very difficult.

TABLE 1.—*Numbers of embryo sacs in ovules of 5 strains of* Pennisetum ciliare *and 1 strain of* Cenchrus setigerus

Strain	Ovules per indicated number of embryo sacs								Mean
	1	2	3	4	5	6	7	8	
P.I. 185641	0	3	15	22	18	11	7	2	4.6
P.I. 156546	6	13	17	12	3	1	0	0	2.9
Puerto Rican	3	12	10	7	2	0	0	0	2.8
P.I. 193445	8	16	19	4	3	0	0	0	2.6
P.I. 164414[1]	14	17	11	5	0	0	0	0	2.2
P.I. 202513	22	7	2	2	0	0	0	0	1.5

[1] *Cenchrus setigerus.*

As an alternative technique in studies to determine the role of pollination and fertilization in caryopsis development, entire stigmas were removed from florets at given intervals before and after anthesis. Ovaries from which the stigmas were removed within several hours before or after anthesis remained turgid and normal in appearance for as long as 48 hours and were apparently not damaged by the treatment. Moreover, removal of the stigmas at somewhat later stages did not affect seed set adversely.

Data on the relation between removal of the stigmas at various times and subsequent seed set are given in table 2 for two strains of *P. ciliare.* The results obtained in these strains, as well as less complete data obtained from a plant of P.I. 164414 that was later referred to *C. setigerus,* are closely comparable; although normal seed set in the various strains studied differs appreciably. Removal of the stigmas before anthesis or within 2½ hours after anthesis effectively eliminates seed production, presumably because growth of the pollen tube into the ovule is precluded. The formation of low percentages of seeds in treatments made during this interval probably re-

flects faulty technique, *e.g.*, incomplete removal of the stigmas from some florets. Removal of the stigmas 3 to 3½ hours after anthesis gives results intermediate between the earlier treatments and normal seed set. This result probably reflects the fact that some of the pollen tubes have completed their growth through the tissue of the stigmas by this time. Removal of the stigmas later than 4 hours after anthesis had no effect on seed set, the percentages of seeds produced in these cases being essentially the same as those obtained from samples of florets of the same age from which

TABLE 2.—*Seeds set in florets of 2 strains of* Pennisetum ciliare *from which the stigmas were removed at given intervals before and after anthesis*

Strain	Treatment: Stigmas removed—	Florets treated	Seeds set	
			Number	Percent
P.I. 185641	36 hrs. before anthesis	101	2	2.0
	0 to ½ hr. after anthesis	115	1	.9
	1 to 1½ hrs. after anthesis	188	7	3.7
	2 to 2½ hrs. after anthesis	115	2	1.7
	3 to 3½ hrs. after anthesis	121	35	28.9
	4 to 4½ hrs. after anthesis	124	112	90.2
	5 to 5½ hrs. after anthesis	99	87	87.9
	Control (stigmas not removed)	284	243	85.6
P.I. 193445	36 hrs. before anthesis	104	2	1.9
	0 to ½ hr. after anthesis	261	3	1.1
	1 to 1½ hrs. after anthesis	109	0	0
	2 to 2½ hrs. after anthesis	86	0	0
	3 to 3½ hrs. after anthesis	106	7	6.6
	4 to 4½ hrs. after anthesis	121	32	26.4
	5 to 5½ hrs. after anthesis	114	39	34.2
	Control (stigmas not removed)	352	112	31.8

the stigmas had not been removed. The results obtained in this material are very similar to those reported by Warmke (6) for *Panicum maximum*.

The cytological examination of a large number of ovules, most of which were collected 8 to 12 hours after anthesis to obtain early endosperm divisions, revealed evidence of the growth of pollen tubes into one or more embryo sacs in a large majority of the ovules. Penetration of an embryo sac by a pollen tube is characteristically evidenced by a mass of cytoplasm intruded into the embryo sac, in the vicinity of the egg cell. In many embryo sacs, one or both synergids are crushed by entry of the pollen tube, although the nuclei of the synergids are ephemeral and usually have disappeared by the time the pollen tube enters the embryo sac. The deposition of dark-staining granules on the surface of the egg cell, associated with penetration

of the pollen tube in *Panicum maximum* (6), was not found in the present material. An additional indication of the necessity of pollination, and presumably fertilization, for development of the caryopsis is found in the fact that neither the egg nor the polar nucleus divides in florets from which the stigmas have been removed at anthesis.

Only two embryo sacs containing early endosperm divisions in which the chromosome complement could be counted were found in several hundred ovules sectioned for this purpose. One of the division figures had 54 chromosomes (fig. 2, I), and the second, although the chromosomes could not be counted with certainty, had approximately that number. Both of these division figures were found in embryo sacs that originally had been 4-nucleate, as indicated by the absence of antipodal tissue, which persists until after fertilization in 8-nucleate embryo sacs. Thirty-six of the chromosomes in the dividing endosperm nuclei observed apparently were derived from an unreduced polar nucleus, and the remaining 18 chromosomes from a normal, reduced sperm nucleus.

The appearance of the egg cell in most embryo sacs containing 2-, 4- or 8-nucleate endosperms (fig. 2, G) strongly suggests that the egg usually is not fertilized—nor would such an event be expected. Division of the egg apparently is stimulated by development of the endosperm, and ordinarily takes place after the endosperm has reached a 16- to 32-nucleate condition (fig. 2, H). Occasionally, however, a multicellular embryo was seen where there was no endosperm development, either in the particular embryo sac or in other embryo sacs within the ovule. Various other infrequently encountered aberrations at this stage include development of a massive endosperm tissue with no division of the associated egg, and such phenomena in ovules containing multiple embryo sacs as development of a multicellular embryo not associated with any endosperm tissue in one embryo sac and an extensive endosperm associated with an individed egg cell in another embryo sac in the same ovule.

POLYEMBRYONY

In ovules containing several embryo sacs, some of the embryo sacs usually are located in a manner that makes their penetration by a pollen tube very unlikely. Cytological examination, however, indicates that frequently pollen tubes do grow into two or even three embryo sacs in an ovule. This is particularly true in P.I. 185641, which produces an average of 4.6 embryo sacs per ovule. The examination of later stages indicates the production of two embryos in approximately 20 percent of the ovules in this strain, with an occasional ovule having three embryos. The percentages of ovules containing multiple embryos are much lower in the other strains examined, as also are the average numbers of embryo sacs per ovule (table 1). The ger-

mination of seeds of these strains on moist filter paper gives data which support the cytological observations. Twin seedlings were produced from 17.7 percent of the seeds of P.I. 185641, and from less than 3 percent of the seeds from the other strains involved in the investigation. In P.I. 164414 (*C. setigerus*), cytological examination revealed the formation of two embryos in occasional ovules, but no examples of twinning were found from 80 seeds germinated.

The production of multiple embryos in *P. ciliare* and *C. setigerus* results from the development of egg cells in separate embryo sacs; there is no indication of development of more than one egg in a given embryo sac, as sometimes happens in the production of multiple embryos in *Poa pratensis* (5). The formation of multiple embryos within multiple embryo sacs, involving phenomena closely comparable to those observed in the present material, has been observed in two additional species of *Pennisetum*, *P. setaceum* and *P. villosum* (4). In these species, as in *P. ciliare*, the embryos have a predominantly aposporic origin. The production of both aposporic and sexual embryo sacs in an occasional ovule (fig. 2, D), and the finding of an occasional chalazal megaspore, without enlarging nucellar cells (fig. 1, E), however, suggests the possibility of an infrequent sexual embryo.

DISCUSSION

Recent investigations have established the occurrence of apomictic phenomena in several species of *Pennisetum* and in a single species of *Panicum*, in the grass tribe Paniceae. In a broad survey of reproductive processes in a number of *Pennisetum* species, Narayan (4) has reported that *P. villosum* is an obligate apomict, and that *P. clandestinum*, and possibly *P. orientale* are facultative apomicts. In addition, Narayan found evidence of apospory and possible facultative apomixis in the examination of limited material of *P. ramosum*. Warmke (6) has recently reported the occurrence of facultative apomixis in five agronomic varieties of *Panicum maximum*, with details of the mechanisms involved. After the present study had been completed, Fisher, *et al.* (1), as the result of concurrent investigations, reported evidence for the occurrence of apomixis in *P. ciliare* and *C. setigerus*.

The reproductive behavior observed in the present material exhibits many similarities to that found in *Panicum maximum*. As in the latter species, a striking phenomenon is the production of mature embryo sacs of two distinctly different types; the predominant type being 4-nucleate and the much less frequent type 8-nucleate. *Pennisetum ciliare* and *Cenchrus setigerus* thus provide a fifth and sixth example, with *Pennisetum orientale*, *P. setaceum*, *P. villosum*, and *Panicum maximum*, of the production of mature 4-nucleate embryo sacs outside the family Onagraceae. It is un-

doubtedly significant that all of these species, in three closely related genera, are aposporous and probably also pseudogamous apomicts.

Although it was not possible to determine with certainty whether there was a consistent difference in chromosome number between the two types of embryo sacs in *Panicum maximum* (6), appreciable evidence pointed toward the conclusion that the 4-nucleated type of embryo sac was usually unreduced and the 8-nucleate type was at least sometimes reduced. The incidence of 8-nucleate embryo sacs tends to be lower in *Pennisetum ciliare* than in *Panicum maximum*. Evidence from the examination of hundreds of ovules is consistent with that general conclusion. The apparent infrequent development of chalazal megaspores correlates well with the subsequent low incidence of 8-nucleate embryo sacs. In addition, 11 of the 12 embryo sacs in which chromosome counts could be obtained were unreduced. All of these unreduced embryo sacs in which development had progressed to the 2-nucleate condition had the two nuclei located at one end, i.e., the embryo sacs were apparently destined to be 4-nucleate. It is a matter of appreciable interest that the only 4-nucleate embryo sac in which division was observed contained the reduced number of chromosomes, thus providing critical evidence that at least some of the 8-nucleate embryo sacs are derived from functional megaspores.

SUMMARY

Cytological studies of seven collections of *Pennisetum ciliare* from widely separated locations indicate that apomixis, including apospory and pseudogamy, is the predominant mode of reproduction in this highly variable species. Chromosome numbers of $2n = 43$ and $2n = 48$ were found, in addition to the numbers 32, 36, 40, and 54 previously reported for this species. Meiotic behavior in PMC's varies widely in the different strains, but, in general, is characterized by limited quadrivalent formation and considerable irregularity and chromosome nondisjunction. The products of meiosis in the ovules usually degenerate soon after they are formed, and one or more cells of the nucellus enlarge to become unreduced embryo sacs. Both 4- and 8-nucleate mature embryo sacs are produced, the latter being relatively uncommon, however. Evidence from *Panicum maximum* and the materials involved in the present study strongly suggests that mature 4-nucleate embryo sacs are typically unreduced and that 8-nucleate embryo sacs are typically reduced in these species. Pollination is necessary for the initiation of divisions in the endosperm, which precede the division of the unreduced and unfertilized egg. Closely comparable reproductive behavior was found in material of a single collection of *Cenchrus setigerus*.

RESUMEN

El estudio citológico de especímenes de *Pennisetum ciliare* de siete colecciones procedentes de distintas regiones, reveló que en esta especie tan variable las formas predominantes de reproducción son aposporia y pseudogamia. Además de los números diploides 32, 36, 40, y 54, informados anteriormente, se encontraron también los de 43 y 48. Aunque el proceso meyótico en las células madres es muy distinto en las diferentes variedades el mismo se caracteriza, en términos generales, por pocas agrupaciones cuadrivalentes, considerable irregularidad y no-disyunción cromosómica. Los productos de la meyosis en los óvulos casi siempre degeneran poco después de haberse formado y una o más células de la nucela, al aumentar de tamaño, se convierte en sacos embrionales no reducidos. Se forman dos tipos de sacos embrionales maduros, unos con cuatro núcleos y otros con ocho, pero éstos últimos son relativamente escasos. Este estudio, así como otros practicados con *Panicum maximum*, parecen indicar que en estas dos especies los sacos embrionales maduros dotados de 4 núcleos son normalmente no reducidos, mientras que los de ocho núcleos son reducidos. La polinización es necesaria para que el endosperma comience a dividirse, proceso que antecede a las divisiones que se verifican en el óvulo no reducido y no fecundado. Una forma de reproducción muy parecida a la descrita se observó también en especímenes de una colección de *Cenchrus setigerus*.

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Antigenic Relationship between Puerto Rican Pepper-Mosaic Virus and a Strain of Potato Virus Y

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INTRODUCTION

Puerto Rican pepper-mosaic virus, studied in some detail by Roque and Adsuar (1)[2] was reported by these authors as having properties similar to the potato vein-banding virus. Viruses producing the "mottle" or mosaic symptoms in pepper and tobacco plants (2, 3, 4) have been related at one time or another to viruses producing diseases in the potato, particularly the "Y" group of viruses. Cross-resistance tests involving the potato vein-banding virus, potato virus Y, and the Puerto Rican pepper-mosaic virus have shown a relationship between the two infectious agents.[3] It therefore seemed of interest to confirm the relation between potato virus Y and the Puerto Rican virus by means of precipitin tests.

EXPERIMENTAL PROCEDURE

A typical strain of potato virus Y[4] and the Puerto Rican pepper mosaic virus (P.R.P.M.) were propagated on young tobacco plants, variety Virginia 12. Leaves showing good symptoms were harvested 2 to 3 weeks after inoculation, washed in sterile tapwater, ground in mortars, and the sap expressed by wringing the macerate through two layers of cheesecloth. The sap was then clarified by low-speed centrifugation for 20 to 30 minutes. Each virus was injected separately by the intraperitoneal route into two rabbits. Five-milliliter doses of infective clarified sap were given at 3- to 4-day intervals as immunizing antigen. A total of eight injections was given to each rabbit using always freshly expressed sap. Throughout the immunization period blood samples were taken to ascertain the antibody response.

The precipitation test was employed for determining the antibody response and the relationship between the viruses. The injection of the antigens produced antisera that precipitated with clarified sap from tobacco plants inoculated with both the potato Y virus strain and the P.R.P.M. virus.

The sera were first absorbed with normal tobacco sap and then titrated

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[2] Numbers in parentheses refer to Literature Cited, p. 167.

[3] Unpublished data, J. Adsuar.

[4] We are indebted to A. Frank Ross, College of Agriculture, Cornell University, for supplying this strain.

against both homologous and heterologous antigens. In the tests, dilutions of serum made in 0.85-percent saline solution were mixed with an equal volume of clarified infective sap. The mixtures were shaken and incubated at 37°C. for 2 hours. Readings were made at 1 and 2 hours after incubation.

Cross-absorption tests were carried out to determine whether specific antigenic groupings could be detected in each virus. The absorption technique was as follows: 1 part of undiluted serum was mixed with 9 parts of either homologous or heterologous antigen (infective, clarified sap). The mixtures were incubated for 2 hours at 37°C. and placed in the refrigerator until the following day. The precipitate formed was removed by centrifugation. The absorbed sera were then titrated against the homologous and the heterologous antigens.

TABLE 1.—*Titer of antisera with homologous and heterologous antigen*

Serum	Titer with antigen—	
	P.R.P.M.	Y
Y 890[1]	1/320	1/640
Y 982	1/320	1/320
P.R.P.M. 929[2]	1/1280	1/1280
P.R.P.M. 971	1/320	1/160

[1] Y antisera.
[2] P.R.P.M. antisera.

TABLE 2.—*Cross-absorption of antisera to P.R.P.M. and Y antigens*

Serum	Titer with homologous antigen	Titer after absorption with—	
		Y antigen	P.R.R.M. antigen[2]
Y 890[1]	1/640	0	0
Y 982	1/320	0	0
P.R.P.M. 929[2]	1/1280	1/20	0
P.R.P.M. 971	1/320	0	0

[1] Antisera prepared with Y antigen.
[2] Antisera prepared with P.R.P.M. antigen.
[3] Lowest dilution of serum tested was 1/20 (final dilution).

RESULTS

As can be seen from table 1 the sera reacted almost as strongly with the homologous as with the heterologous antigen. This alone proves that the potato Y and P.R.P.M. viruses are closely related strains. In table 2 the

cross-absorption tests show that the P.R.P.M. antigen removed all antibodies to the Y antigen from the Y antisera. However when P.R.P.M. antiserum 929 was absorbed with Y antigen it still retained some precipitating power for the P.R.P.M. antigen, reacting at a dilution of 1/20 (final dilution). This indicates the presence of a minor antigen in P.R.P.M. virus, which is absent from the Y virus. P.R.P.M. antiserum 971 did not react in the same way as 929, but this may be explained on the basis of its titer being fourfold lower than that of 929. It is well known that weak and strong sera behave differently both qualitatively and quantitatively (5).

SUMMARY

Antisera were prepared against the Puerto Rican pepper-mosaic virus (P.R.P.M.) and a typical strain of potato virus Y by inoculating rabbits with clarified sap from tobacco plants infected separately with each virus. All the four sera thus prepared reacted with both antigens. Cross-absorption tests showed that the P.R.P.M. virus possessed a minor antigenic component not found in the Y virus.

RESUMEN

Se prepararon antisueros para el virus que produce el mosaico del pimiento en Puerto Rico (P.R.P.M.) y una cepa tipo del virus Y que ataca la papa, inoculando conejos con el jugo clarificado de plantas de tabaco infectadas separadamente con cada uno de estos virus. Todos los cuatro sueros, así preparados, reaccionaron con ambos antígenos. Pruebas cruzadas de absorción demostraron que el virus P.R.P.M. posee componentes antigénicos que no se encuentran en el virus Y.

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Reaction of some Nicotiana Species to the Pepper- and Common Tobacco-Mosaic Viruses

J. Adsuar and L. López Matos[1]

INTRODUCTION

As a result of a survey recently conducted with the purpose of determining the viruses attacking the filler-type tobacco grown in Puerto Rico developed that the prevalent viruses on this crop were: Common tobacco mosaic virus, pepper-mosaic virus, and leaf curl virus, in that respective order of importance.

The pepper-mosaic virus was found originally in pepper by Roque and Adsuar, (1)[2] and more recently was shown by Pérez and Adsuar (2) to be serologically identical with the Y (Cornell strain)[3] virus of potato. Pepper mosaic virus is transmissible by mechanical means and also by the green peach aphid, *Myzus persiceae*. The leaf-curl virus has been under study by Bird at this Station and is indistinguishable from the virus causing leaf curl and tobacco Kroepoek, as reported from other parts of the world. Leaf curl is transmitted only by the white fly *Bemisia tobaci* and has been found to cause a mosaic of "tuatúa", *Aderonopium gossypifolium*, a weed present in the Island. However, leaf curl, as far as we have been able to observe, occurs only sporadically and does not as yet present any serious menace to tobacco.

In view of the situation disclosed by the survey it was considered of interest to explore the possibilities offered by testing different varieties and species of Nicotiana with the objective of finding sources of resistance to the viruses prevalent on tobacco in our fields.

METHODS AND MATERIALS

Seed of the different species of Nicotiana were kindly supplied by Lawrence Burke, geneticist, Tobacco Special Crops Section, U.S.D.A. Beltsville, Md. These were planted in sterilized soil and transplanted 3 to 4 weeks later into 5-inch pots filled with sterile soil. The plants were inoculated when about 2 months old by rubbing the leaves, previously dusted with carborundum, with the inoculum. Inoculum was prepared by macerating leaves from tobacco plants infected with either pepper mosaic or common tobacco mosaic, and extracting the juice. In order to check the presence and infectivity of the viruses used, the inoculum was tested on plants of

[1] Pathologist and Assistant Plant Pathologist respectively, Agricultural Experiment Station, University of Puerto Rico, Río Piedras, P. R.

[2] Numbers in parentheses refer to Literature Cited, p. 171.

[3] Kindly supplied by A. F. Ross, Cornell University, Ithaca, N. Y.

tobacco carrying the *nn* factor for the common tobacco mosaic virus, and on Large Bell Hot pepper, already known as a differential host (1) for the pepper-mosaic virus, and in which pepper-mosaic virus causes a character-

TABLE 1.—*Results of inoculating some Nicotiana species with pepper and common tobacco mosaic viruses*[1]

Species	Inoculum		Symptoms		Source of inoculum	
	PMV	TMV	PMV	TMV	PMV	TMV
Nicotiana pauciflora	—	—				
N. glauca	0/8	0/5			*N. tobacum*	*N. tobacum*
N. undulata	7/8	4/4	VC-M-LD	NS	do.	Do.
N. acuminata	8/8	?	N-D		do.	Do.
N. repanda	8/8	6/6	VC-M-C	NS	do.	Do.
N. tomentosiformis	0/8	5/6		VC-C-M	do.	Do.
N. velutina	5/12	3/3	VC-M-C	VC-M-C-LD	do.	Do.
N. clevelandii	4/4	3/3	N-D	VC-C-D	do.	Do.
N. bonariensis	2/3	4/4	VC-C-M	VC-C-M	do.	Do.
N. debneyi	7/8	3/3	VC-C-M	VC-C-M	do.	Do.
N. corymbosa	—	—				
N. angustifolia	1/3	2/2	VC-C-M-N-D	NS	*N. repanda*	*N. tabacum*
N. benthamiana	4/4	2/2	VC-C-M	VC-C	do.	Do.
N. bigelovii	8/8	?	VC-C-M		*N. tabacum*	Do.
N. otophora	0/8	3/3		VC-C-M	*N. repanda*	Do.
N. palmeri	7/7	2/3	VC-C-M	VC-C-M-LD	do.	Do.
N. paniculata	8/8	3/3	VC-C-M	VC-C-M-LD	*N. tabacum*	Do.
N. occidentalis	1/1	1/1	VC-M	VC-M	do.	Do.
N. longiflora	7/8	3/3	VC-M-C	VC-C	do.	Do.
N. megalosiphon	8/8	3/3	VC-C-M	VC-C-M	do.	Do.
N. maritima	2/2	2/2	VC-C-M	VC-C-M-LD	do.	Do.
N. gossei	8/8	6/6	VC-M	NS	do.	Do.
N. goodspedii	7/7	3/3	VC-M	NS	do.	Do.
N. glutinosa	7/8	1/1	VC-C-M	NS	do.	Do.

[1] Numerator—plant infected; denominator—plants inoculated. PMV = pepper mosaic virus; TMV = tobacco mosaic virus; — = seed did not germinate; ? = no plants available for testing; VC = vein clearing; M = mottling; N = necrosis; NS = localized necrotic spots only; C = chlorosis; LD = leaf deformation; D = death of plant.

istic systemic vein necrosis, defoliation, and death. All inoculated plants were kept in the greenhouse held at a temperature of 70° to 98°F. All trials were conducted between November 1954 and January 1955.

RESULTS

The results of the inoculation performed on the different Nicotiana species are presented in table 1. Some of the species refused to germinate; others did

so poorly. This accounts for the very limited number of plants inoculated in some of the tests.

Of the 24 species tested, *N. glauca*, *N. tomentosiformis*, and *N. otophora* were found to remain symptomless when inoculated with the pepper-mosaic virus. As shown in table 1, *N. glauca* was the only one of the species showing resistance to both viruses.

Attempts were made to determine the presence of pepper-mosaic virus in symptomless *N. glauca*, *N. tomentosiformis*, and *N. otophora*, by extracting leaf juice and inoculating back into the Large Bell Hot pepper. *N. glauca* was found free from pepper virus in all cases. *N. tomentosiformis* juice caused mottling and chlorosis in some cases, but no systemic vein necrosis in the Large Bell Hot. Only one *N. otophora* of several plants tested produced systemic vein necrosis in the differential host.

CONCLUSION

The survey conducted during the past 2 years throughout the filler-type tobacco regions of the Island clearly indicated that the two most important and prevalent viruses are the common tobacco- and pepper-mosaic viruses. There are already in existence several mosaic-resistant tobacco varieties, but we know very little about their adaptability to our conditions. We know of no other source of resistance to the pepper mosaic except the species reported in this paper. Although the possibility still exists of finding better sources of resistance in the genus Nicotiana against the two viruses already mentioned, we consider that efforts should be made to incorporate, if possible, the resistance shown by the above-mentioned Nicotiana species into our best commercial tobacco types in order to produce resistant varieties better adapted to our conditions.

RESUMEN

Como resultado de un censo verificado con el propósito de determinar los virus predominantes en el tabaco de tripa (filler tobacco) que se cultiva en Puerto Rico, se encontró que los dos más importantes son el causante del mosaico común y el del mosaico del pimiento de Puerto Rico.

Se inocularon 24 especies de Nicotiana para determinar su resistencia a estos dos virus.

Se encontró que las especies *N. glauca*, *N. tomentosiformis*, y *N. otophora* no presentan síntomas al ser inoculadas con el virus causante del mosaico del pimiento de Puerto Rico.

La *N. glauca* es aparentemente resistente a ambos virus.

Por lo tanto se aconseja tratar de cruzar especialmente la *N. glauca* con

nuestros mejores tabacos para incorporarle a estos la resistencia adecuada a los dos virus arriba mencionados.

LITERATURE CITED

1. Roque, A., and Adsuar, J., Studies on the mosaic of peppers (*Capsicum frutescens*) in Puerto Rico, *J. Agr. Univ. P. R.* **25** (4) 40–50, 1941.
2. Pérez Otero, E., and Adsuar, J., The antigenic relationship between the P. R. pepper-mosaic virus and potato virus Y, *J. Agr. Univ. P. R.* **39** (3) 165–7, 1955.

RESEARCH NOTES

CHEMICAL ANALYSIS OF STONE CELLS PRESENT IN GUAVA

The guava pulp was dispersed in water and the stone cells were removed by centrifugation. The results of a complete analysis of four different samples of stone cells, expressed in percentage, are as follows:

Constituent	*Sample 1*	*Sample 2*	*Sample 3*	*Sample 4*
Ashes	0.66	0.45	0.56	0.46
Protein	3.54	3.39	4.66	3.46
Lignin	37.42	38.56	42.72	41.80
Fat	.35	.24	.42	.55
Cellulose	59.45	56.37	52.42	54.32
Crude fiber	56.60	60.70	55.00	56.40

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Corrigendum

In the paper by J. Ives Townsend and Marshall R. Wheeler entitled "Notes on Puerto Rican Drosophilidae, including descriptions of two new species of Drosophila", appearing in this journal, April 1955, the following correction is to be made in the 4th line of p. 62:
Read: "2 pairs of V's" for "2 pairs of U's."

A NEW HOST OF THE VIRUS (VIRUS B) CAUSING CUCUMBER MOSAIC IN PUERTO RICO

Cucumber mosaic in Puerto Rico is caused by two different viruses[1] which have been tentatively named A and B. These viruses differ from each other in symptomatology, host range, and physical properties. As far as we have been able to determine virus A is not transmissible to plants outside the cucurbit family. On the other hand, virus B is capable of infecting not only cucurbits but plants of other families as well.

The purpose of this note is to report the presence of a mosaic on *Impatiens Sultani* Hook. caused by a virus which, when inoculated on cucumber and *Phytolacca decandra* Linn., produces symptoms indistinguishable from those produced by virus B on these hosts. *Impatiens sultani* Hook. is an ornamental known locally as *Alegría* and belongs to the family Balsaminaceae. The infected plants were found on the Station grounds.

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[1] Adsuar, J. and Cruz Miret, A., Virus Diseases of Cucumbers in Puerto Rico' Tech. Paper No. 6, Agr. Exp. Sta., Univ. P. R., 1950.